

persisted after controlling for nutritional variables (blood iron [Fe], calcium [Ca²⁺], copper [Cu], magnesium [Mg], and zinc [Zn]). This observation may be explained by lower GI absorption of Pb ingested with or in close temporal proximity to meals. Direct evidence for meals lowering GI absorption of Pb has also been reported for adults ([Maddaloni et al., 1998](#); [James et al., 1985](#)).

Nutritional interactions of Pb with dietary elements (e.g., Fe, Ca²⁺, Zn) are complex. Pb competes with other elements for transport and binding sites that can result in adjustments of homeostatic regulators to absorb and retain needed elements. Additionally, low levels of macronutrients may alter Pb bioaccessibility in the GI tract. Genetic variation in absorption and metabolism may modify all of the above.

Children who are Fe-deficient have higher blood Pb concentrations than similarly exposed Fe-replete children, suggesting that Fe deficiency may result in higher Pb absorption or, possibly, other changes in Pb biokinetics that contribute to altered blood Pb concentrations ([Schell et al., 2004](#); [Marcus and Schwartz, 1987](#); [Mahaffey and Annett, 1986](#)). Studies conducted in animal models have provided direct evidence for interactions between Fe deficiency and increased Pb absorption, perhaps by enhancing binding of Pb to Fe-binding proteins in the intestine ([Bannon et al., 2003](#); [Morrison and Quarterman, 1987](#); [Barton et al., 1978b](#)). An analysis of data from a sample 448 woman (age 20-55 years) did not find a significant association between Fe body stores (indicated from serum ferritin concentration) and blood Pb concentrations, although depleted iron stores (serum ferritin of <12 µg/L) was associated with higher blood concentrations of Cd, cobalt (Co) and manganese (Mn) higher ([Meltzer et al., 2010](#)). The effects of Fe nutritional status on blood Pb include changes in blood Pb concentrations in association with genetic variation in genes involved in Fe metabolism. For example, genetic variants in the hemochromatosis (HFE) and transferrin genes are associated with higher blood Pb concentrations in children ([Hopkins et al., 2008](#)). In contrast, HFE gene variants are associated with lower bone and blood Pb levels in elderly men ([Wright et al., 2004](#)).

Several studies have suggested that dietary Ca²⁺ may have a protective role against Pb by decreasing absorption of Pb in the GI tract and by decreasing the mobilization of Pb from bone stores to blood. 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 Ca²⁺ carbonate (0.2 g Ca²⁺ carbonate) than when the Pb was ingested without additional Ca²⁺ ([Blake and Mann, 1983](#); [Heard and Chamberlain, 1982](#)). A similar effect of Ca²⁺ occurs in rats ([Barton et al., 1978a](#)). Similarly, an inverse relationship was observed between dietary Ca²⁺ intake and blood Pb concentration in children, suggesting that children who are Ca²⁺-deficient may absorb more Pb than Ca²⁺-replete children ([Elias et al., 2007](#); [Schell et al., 2004](#); [Mahaffey et al., 1986](#); [Ziegler et al., 1978](#)). These

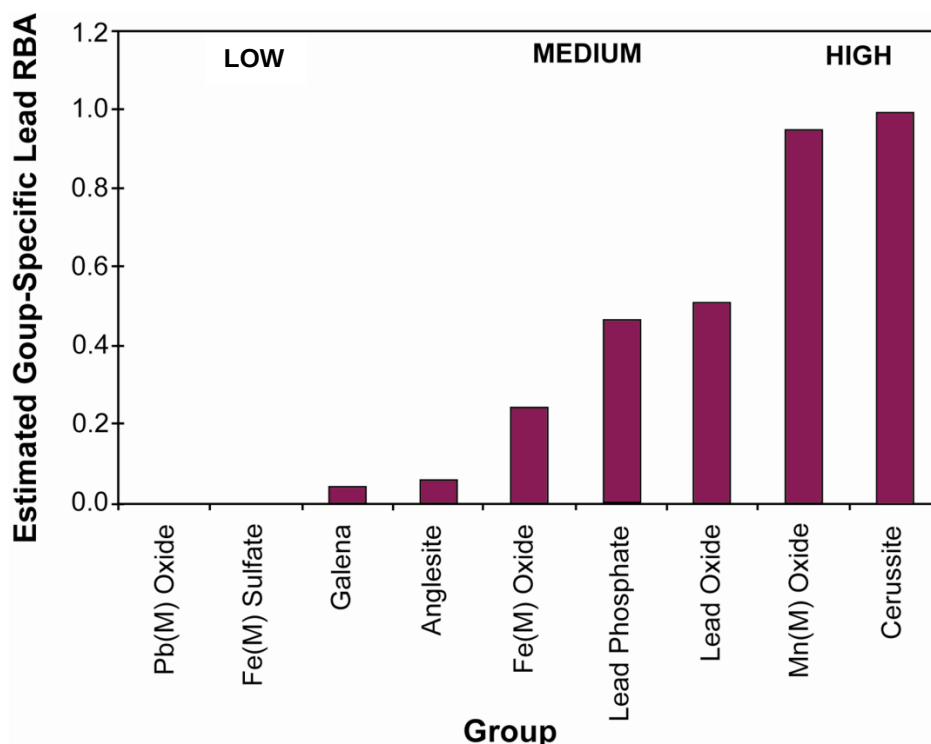
observations suggest that Ca^{2+} and Pb share and may compete for common binding and transport mechanisms in the small intestine which are regulated in response to dietary Ca^{2+} and Ca^{2+} -body stores ([Fullmer and Rosen, 1990](#); [Bronner et al., 1986](#)). However, animal studies have also shown that multiple aspects of Pb toxicokinetics are affected by Ca^{2+} nutritional status. For example, feeding rats a Ca^{2+} deficient diet is associated with increased Pb absorption, decreased whole body Pb clearance, and increased volume of distribution of Pb ([Aungst and Fung, 1985](#)). These studies suggest that associations between Ca^{2+} nutrition and blood Pb that have been observed in human populations may not be solely attributable to effects of Ca^{2+} nutrition on Pb absorption. Other potential mechanisms by which Ca^{2+} nutrition may affect blood Pb and Pb biokinetics include effects on bone mineral metabolism and renal function.

Blood Pb concentrations in young children have also been shown to increase in association with lower dietary Zn levels ([Schell et al., 2004](#)). Mechanisms for how Zn affects blood Pb concentration, i.e., whether it involves changes in absorption or changes in distribution and/or elimination of Pb, have not been determined.

Dissolution of Pb from the soil/mineralogical matrix in the stomach appears to be the major process that renders soil Pb bioaccessible for absorption in the GI tract. Absorption of Pb has been shown to vary depending upon the Pb mineralogy and physical characteristics of the Pb in the soil (e.g., encapsulated or exposed) and size of the Pb-bearing grains. GI absorption of larger Pb-containing particles ($>100\ \mu\text{m}$) tends to be lower than smaller particles ([Healy et al., 1992](#); [Barltrop and Meek, 1979](#)). Absorption of Pb in soils and dust has been most extensively studied in the in vivo swine model. Gastric function of swine is thought to be sufficiently similar to that of humans to justify use of swine as a model for assessing factors that may affect GI absorption of Pb from soils in humans ([Juhasz et al., 2009](#); [U.S. EPA, 2007b](#); [Casteel et al., 2006](#); [Casteel et al., 1997](#); [Weis and Lavelle, 1991](#)). Other practical advantages of the swine model over rodent models have been described, and include: absence of coprophagia; ease with which Pb dosing can be administered and controlled; and higher absorption fraction of soluble Pb (e.g., Pb acetate) in swine, which is more similar to humans than rats ([Smith et al., 2009a](#)). The swine studies measure blood and/or tissue Pb (e.g., kidney, liver, bone) concentrations following oral dosing of swine with either soil or with a highly water soluble and fully bioaccessible form of Pb (e.g., Pb acetate). A comparison of the internal concentrations of Pb under these two conditions provides a measure of the bioavailability (i.e., absorption) of Pb in soil relative to that of Pb acetate, which is typically referred to as relative bioavailability (RBA). Relative bioavailability measured in the swine assay is equivalent to the ratio of the absorbed fraction (AF) of ingested dose of soil Pb to that of water-soluble Pb acetate (e.g., $\text{RBA} = \text{AF}_{\text{Soil Pb}}/\text{AF}_{\text{Pb acetate}}$).

Collectively, published studies conducted in swine have provided 39 estimates of Pb RBA for 38 different soil or “soil-like” test materials ([Bannon et al., 2009](#); [Smith et al., 2009a](#); [Casteel et al., 2006](#); [Marschner et al., 2006](#)). The mean of RBA estimates from 25 soils is 0.49 (± 0.29 [SD]), median is 0.51, and 5th to 95th percentile range is 0.12 to -0.89. RBA estimates for soils collected from 8 firing ranges were approximately 1.0 ([Bannon et al., 2009](#)). The relatively high RBA for the firing range soils may reflect the high abundance of relatively un-encapsulated Pb carbonate (30-90% abundance) and Pb oxide (1-60%) in these soils. Similarly, a soil sample (low Pb concentration) mixed with a NIST paint standard (55% Pb carbonate, 44% Pb oxide) also had a relatively high bioavailability (0.72) ([Casteel et al., 2006](#)). Samples of smelter slag, or soils in which the dominant source of Pb was smelter slag, had relatively low RBA (0.14 – 0.40, n = 3), as did a sample from a mine tailings pile (RBA = 0.06), and a sample of finely ground galena mixed with soil ([Casteel et al., 2006](#)).

Based on data for 18 soil materials assayed in swine, RBA of Pb mineral phases were categorized into “low” (<0.25 [25%]), “medium” (0.25-0.75 [25 to 75%]), and “high” (>0.75 [75%]) categories ([Casteel et al., 2006](#)). [Figure 3-3](#) shows some of the materials that fall into these three categories. Mineral phases observed in mineralogical wastes can be expected to change over time (i.e., weathering), which could change the RBA over time. The above observations in swine are supported by various studies conducted in rats that have found RBA of Pb in soils to vary considerably and to be less than 1.0 ([Smith et al., 2009a](#), [2008](#); [Freeman et al., 1996](#); [Freeman et al., 1994](#); [Freeman et al., 1992](#)).



Note: based on results from juvenile swine assays.

Source: Casteel et al. (2006).

Figure 3-3 Estimated relative bioavailability (RBA, compared to Pb acetate) of ingested Pb in mineral groups.

Drexler and Brattin (2007) developed an in vitro bioaccessibility (IVBA) assay for soil Pb that utilizes extraction fluid comprised of glycine, deionized water, and hydrochloric acid at a pH of 1.50 that is combined with sieved test material (<250 µm) for 1 hour. The assay was tested for predicting in vivo RBA of 18 soil-like test materials that were assayed in a juvenile swine assay (Casteel et al., 2006). A regression model relating IVBA and RBA was derived based on these data (Equation 3-1):

$$\text{RBA} = (0.878 \times \text{IVBA}) - 0.028$$

Equation 3-1

where RBA and IVBA are expressed as fractions (i.e., not as percent). The weighted r^2 for the relationship (weighted for error in the IVBA and RBA estimates) was 0.924 ($p < 0.001$). The IVBA assay reported in Drexler and Brattin (2007) has been identified by the U.S. EPA as a validated method for predicting RBA of Pb in soils for use in risk

assessment ([U.S. EPA, 2007e](#)). A review of soil Pb RBA estimates made using the IVBA assay described above and Equation 3-1 identified 270 estimates of Pb RBA in soils obtained from 11 hazardous waste sites. The mean for the site-wide RBA estimates (n = 11 sites) was 0.57 (SD 0.15), median was 0.63, and 5th to 95th percentile range was 0.34 to 0.71.

Equation 3-1 cannot be reliably extrapolated to other in vitro assays that have been developed for estimating Pb bioaccessibility without validation against in vivo RBA measurements made on the same test materials. Comparisons of outcomes among different in vitro assays applied to the same soil test materials have found considerable variability in IVBA estimates ([Juhasz et al., 2011](#); [Smith et al., 2011](#); [Saikat et al., 2007](#); [Van de Wiele et al., 2007](#)). This variability has been attributed to differences in assay conditions, including 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). Smith et al. ([2011](#)) found that algorithms for predicting RBA, based on two different IVBA assays, did not yield similar predictions of RBA when applied to the same material. Given the dependence of IVBA outcomes on assay conditions, in vitro assays used to predict in vivo RBA should be evaluated against in vivo RBA estimates to quantitatively assess uncertainty in RBA predictions ([U.S. EPA, 2007e](#)).

Absorption of Pb in house dusts has not been rigorously evaluated quantitatively in humans or in experimental animal models. The RBA for paint Pb mixed with soil was reported to be approximately 0.72 (95% CI: 0.44, 0.98) in juvenile swine, suggesting that paint Pb dust reaching the gastrointestinal tract maybe highly bioavailable ([Casteel et al., 2006](#)). The same material yielded a bioaccessibility value (based on IVBA assay) of 0.75 ([Drexler and Brattin, 2007](#)), which corresponds to a predicted RBA of 0.63, based on Equation 3-1. A review of indoor Pb RBA estimates made using the IVBA assay and Equation 3-1 identified 100 estimates of Pb RBA in dusts obtained from two hazardous waste sites. Mean Pb RBAs for the Herculaneum site were 0.47 (SD 0.07, 10 samples) for indoor dust and 0.69 (SD 0.03, 12 samples) for soil. At the Omaha site, mean Pb RBAs were 0.73 (SD 0.10, 90 samples) for indoor dust and 0.70 (SD 0.10, 45 samples) for soil. Yu et al. ([2006](#)) applied an IVBA method to estimate bioaccessibility of Pb in house dust samples collected from 15 urban homes. Homes were selected for inclusion in this study based on reporting to the state department of health of at least one child with a blood Pb concentration >15 µg/dL and Pb paint dust may have contributed to indoor dust Pb. The mean IVBA was 0.65 (SD 0.08, age: 52.5 to 77.2 months).

The above results, and the IVBA assays used in studies of interior dust, have not been evaluated against in vivo RBA estimates for dust samples. Although, expectations are

that a validated IVBA methodology for soil would perform well for predicting RBA of interior dust, this validation has not actually been experimentally confirmed. Factors that may affect in vivo predictions of RBA of interior dust Pb could include particle size distribution of interior dust Pb and the composition of the dust matrix, which may be quite different from that of soil.

Other estimates of bioaccessibility of Pb in house dusts have been reported, based on results from in vitro extraction assays that have not been validated for predicting in vivo bioavailability. Bioaccessibility assays that sequentially extract soil at gastric pH followed by intestinal pH tend to show higher bioaccessibility of soil and dust Pb when incubated at gastric conditions ([Juhasz et al., 2011](#); [Lu et al., 2011](#); [Smith et al., 2011](#); [Roussel et al., 2010](#); [Yu et al., 2006](#)). Yu et al. (2006) dissolved Pb dust, obtained from vacuuming carpet samples into simulated gastric and intestinal acids (also [Section 3.1.3.2](#)). The carpet samples were obtained from homes located in northern New Jersey. Pb concentration in carpet ranged from 209 to 1,770 mg/kg dust, with 52-77% of Pb dissolving in simulated gastric acid and 5-32% dissolving in simulated intestinal acids. In a similar test in the U.K., Turner and Simmonds (2006) observed median Pb dust concentrations of 178 mg/kg with approximately 80% bioaccessibility in simulated gastric acid. Jin et al. (2005) observed that bioaccessibility of Pb in soil was proportional to the soil acidity and organic matter content of the soil.

3.2.2 Distribution and Metabolism

A simple conceptual representation of Pb distribution is that it contains a fast turnover pool, comprising mainly soft tissue, and a slow pool, comprising mainly skeletal tissues ([Rabinowitz et al., 1976](#)). The highest soft tissue concentrations in adults occur in liver and kidney cortex ([Gerhardsson et al., 1995](#); [Oldereid et al., 1993](#); [Gerhardsson et al., 1986](#); [Barry, 1975](#); [Gross et al., 1975](#)). Pb in blood (i.e., plasma) exchanges with both of these compartments.

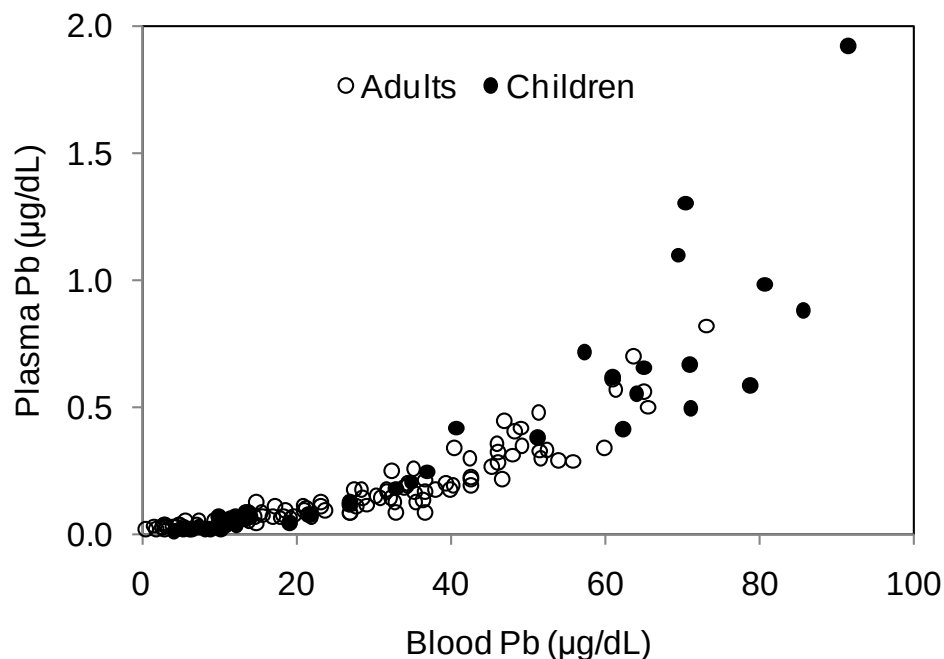
3.2.2.1 Blood

Blood comprises ~1% of total Pb body burden. Pb in blood is found primarily (>99%) in the RBCs ([Smith et al., 2002](#); [Manton et al., 2001](#); [Bergdahl et al., 1999](#); [Bergdahl et al., 1998](#); [Hernandez-Avila et al., 1998](#); [Bergdahl et al., 1997a](#); [Schutz et al., 1996](#)). δ -aminolevulinic acid dehydratase (ALAD) is the primary binding ligand for Pb in erythrocytes ([Bergdahl et al., 1998](#); [Xie et al., 1998](#); [Bergdahl et al., 1997a](#); [Sakai et al., 1982](#)). Two other Pb-binding proteins have been identified in the RBC, a 45 kDa protein

($K_{\max}$ 700 $\mu\text{g/dL}$; K_d 5.5 $\mu\text{g/L}$) and a smaller protein band having a molecular weight of <10 kDa ([Bergdahl et al., 1998](#); [Bergdahl et al., 1997a](#); [Bergdahl et al., 1996](#)). Of the three principal Pb-binding proteins identified in RBCs, ALAD has the strongest affinity for Pb ([Bergdahl et al., 1998](#)) and appears to dominate the ligand distribution of Pb (35 to 84% of total erythrocyte Pb) at blood Pb levels below 40 $\mu\text{g/dL}$ ([Bergdahl et al., 1998](#); [Bergdahl et al., 1996](#); [Sakai et al., 1982](#)). Pb binding to ALAD is saturable; the binding capacity was estimated to be ~ 850 $\mu\text{g/dL}$ RBCs (or ~ 40 $\mu\text{g/dL}$ whole blood) and the apparent dissociation constant has been estimated to be ~ 1.5 $\mu\text{g/L}$ ([Bergdahl et al., 1998](#)). Hematocrit is somewhat higher in the neonate at birth (51%) than in later infancy (35% at 6 months), which may lead to a decrease in the total binding capacity of blood over the first 6 months of life that results in a redistribution of Pb among other tissues ([Simon et al., 2007](#)).

Saturable binding to RBC proteins contributes to an increase in the plasma/blood Pb ratio with increasing blood Pb concentration and curvature to the blood Pb–plasma Pb relationship ([Rentschler et al., 2012](#); [Kang et al., 2009](#); [Jin et al., 2008](#); [Barbosa et al., 2006a](#); [Smith et al., 2002](#); [Manton et al., 2001](#); [Bergdahl et al., 1999](#); [Bergdahl et al., 1998](#); [Bergdahl et al., 1997b](#); [DeSilva, 1981](#)). An example of this is shown in [Figure 3-4](#). Saturable binding of Pb to RBC proteins has several important consequences. As blood Pb increases and the higher affinity binding sites for Pb in RBCs become saturated, a larger fraction of the blood Pb is available in plasma to distribute to brain and other Pb-responsive tissues. This change in distribution of Pb contributes to a curvature in the relationship between Pb intake (at constant absorption fraction) and blood Pb concentration. Plasma Pb also exhibits faster kinetics. Following exposures of 5 adults that resulted in relatively high blood Pb concentrations (56–110 $\mu\text{g/dL}$), the initial (fast-phase) elimination half-time for plasma Pb (38 ± 20 [SD] days) was approximately half that of blood (81 ± 25 days) ([Rentschler et al., 2012](#)).

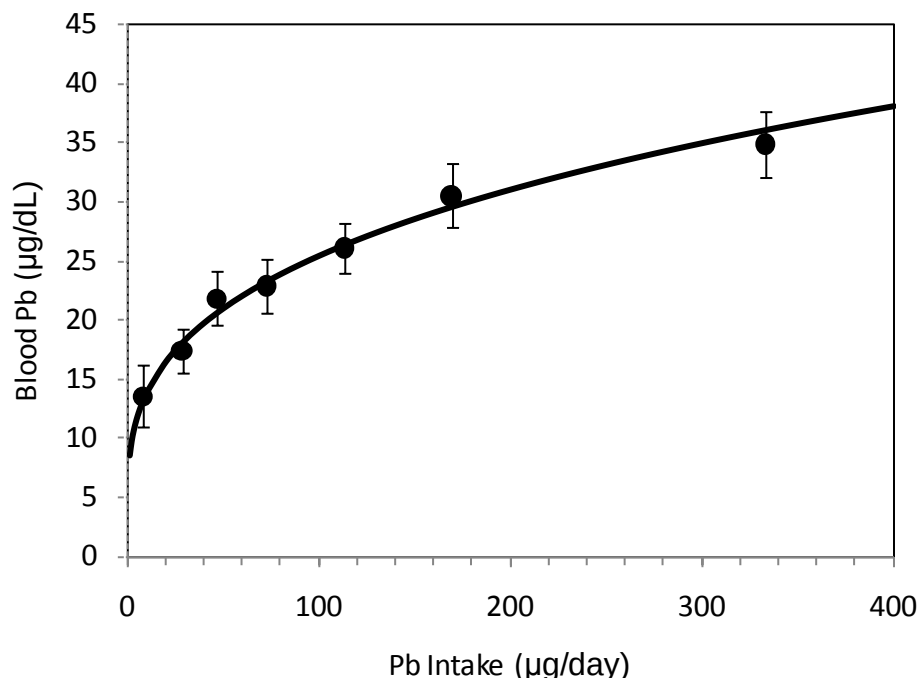
Typically, at blood Pb concentrations <100 $\mu\text{g/dL}$, only a small fraction ($<1\%$) of blood Pb is found in plasma ([Marcus, 1985](#); [Manton and Cook, 1984](#); [DeSilva, 1981](#)). However, as previously noted, plasma Pb may be the more biologically labile and toxicologically effective fraction of the circulating Pb. Approximately 40–75% of Pb in the plasma is bound to proteins, of which albumin appears to be the dominant ligand ([Al-Modhefer et al., 1991](#); [Ong and Lee, 1980a](#)). Pb in serum that is not bound to protein exists largely as complexes with low molecular weight sulfhydryl compounds (e.g., cysteine, homocysteine) and other ligands ([Al-Modhefer et al., 1991](#)).



Source: Adapted with permission of Elsevier Publishing and the Finland Institute of Occupational Health, Bergdahl et al. ([1999](#); [1997b](#)).

Figure 3-4 Plot of blood and plasma Pb concentrations measured in adults and children.

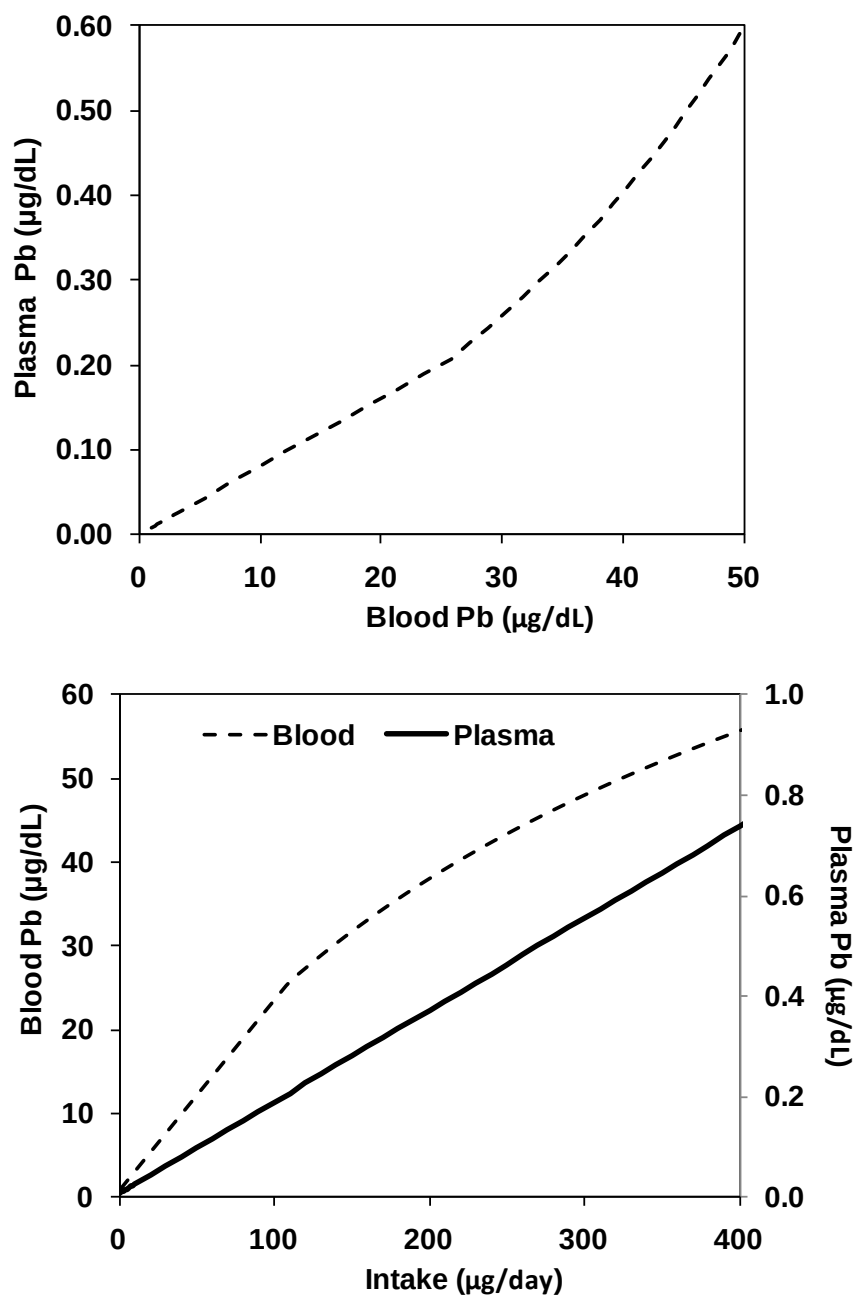
As shown in [Figure 3-4](#), the limited binding capacity of Pb binding proteins in RBCs produces a curvilinear relationship between blood and plasma Pb concentration. The limited binding capacity of RBC binding proteins also confers, or at least contributes, to a curvilinear relationship between Pb intake and blood Pb concentration. A curvilinear relationship between Pb intake and blood Pb concentration has been observed in children ([Sherlock and Quinn, 1986](#); [Lacey et al., 1985](#); [Ryu et al., 1983](#)). As shown in [Figure 3-5](#), the relationship becomes pseudo-linear at relatively low daily Pb intakes (i.e., <10 µg/kg per day) and at blood Pb concentrations <25 µg/dL.



Data represent mean and standard errors for intake; the line is the regression model (blood Pb = $3.9 + 2.43 (\text{Pb intake } [\mu\text{g}/\text{week}]^{1/3})$).
Source: Adapted with permission of Taylor & Francis Publishing, Sherlock and Quinn ([1986](#)).

Figure 3-5 Relationship between Pb intake and blood Pb concentration in infants (N = 105, age 13 weeks, formula-fed).

[Figure 3-6](#) shows the predicted relationship between quasi-steady state blood and plasma Pb concentrations in a 4-year old child using the ICRP model [([Pounds and Leggett, 1998](#); [ICRP, 1994](#); [Leggett, 1993](#)), see [Section 3.3](#) for a brief description of the ICRP model]. The abrupt inflection point that occurs at approximately 25 µg/dL blood Pb is an artifact of the numerical approach to simulate the saturation of binding using discontinuous first-order rate constants for uptake and exit of Pb from the RBC. A continuous function of binding sites and affinity, using empirical estimates of both parameters, yield a similar but continuous curvature in the relationship ([Bergdahl et al., 1998](#); [O'Flaherty, 1995](#)). Nevertheless, either approach predicts a pseudo-linear relationship at blood Pb concentrations below approximately 25 µg/dL which, in this model, corresponds to an intake of approximately 100 µg/day (absorption rate ≈ 30 µg/day) (upper panel). An important consequence of the limited Pb-binding capacity of RBC proteins is that the plasma Pb concentration will continue to grow at a linear rate above the saturation point for RBC protein binding. One implication of limited binding capacity is that a larger fraction of the Pb in blood will become available to distribute to brain and other Pb-responsive tissues as blood Pb increases. This process could potentially contribute to non-linearity in dose-response relationships in studies in which blood Pb is the used as the internal dose metric.



Note: Model simulations are for a 4-year old having from birth a constant Pb intake of between 1 and 400 µg/day. Simulation based on ICRP Pb biokinetics model ([Leggett, 1993](#)).

Figure 3-6 Simulation of quasi-steady state blood and plasma Pb concentrations in a child (age 4 years) associated with varying Pb ingestion rates.

Studies conducted in swine provide additional evidence in support of RBC binding kinetics influencing distribution of Pb to tissues. In these studies, the relationship between the ingested dose of Pb and tissue Pb concentrations (e.g., liver, kidney, bone) was linear, whereas, the relationship between dose and blood Pb was curvilinear with the slope decreasing as the dose increased ([Casteel et al., 2006](#)). Saturable binding of Pb to RBC proteins also contributes to a curvilinear relationship between blood Pb and both plasma Pb and urinary Pb, whereas Pb in plasma and urine are linearly related ([Bergdahl et al., 1997b](#)).

3.2.2.2 Bone

The dominant compartment for Pb in the body is in bone. In human adults, more than 90% of the total body burden of Pb is found in the bones, whereas bone Pb accounts for just under 60% of the body burden in infants less than a year old and just over 70% of the body burden in older children ([Barry, 1975](#)). Bone is comprised of two main types, cortical (or compact) and trabecular (or spongy or cancellous). The proportion of cortical to trabecular bone in the human body varies by age, but on average is about 80 to 20 percent ([O'Flaherty, 1998](#); [Leggett, 1993](#); [ICRP, 1973](#)).

The exchange of Pb from plasma to the bone surface is a rapid process (i.e., adult $t_{1/2}$ = 0.19 and 0.23 hours for trabecular and cortical bone, respectively) ([Leggett, 1993](#)). Some Pb diffuses from the bone surface to deeper bone regions (adult $t_{1/2}$ = 150 days) where it is relatively inert (in adults) and part of a “nonexchangeable” (removed only through bone resorption/remodeling) pool of Pb in bone ([Leggett, 1993](#)).

Pb distribution in bone includes uptake into cells that populate bone (e.g., osteoblasts, osteoclasts, osteocytes) and exchanges with proteins and minerals in the extracellular matrix ([Pounds et al., 1991](#)). 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 ([Meirer et al., 2011](#); [Brès et al., 1986](#); [Miyake, 1986](#); [Verbeeck et al., 1981](#)). Several intracellular kinetic pools of Pb have been described in isolated cultures of osteoblasts and osteoclasts which appear to reflect physiological compartmentalization within the cell, including membranes, mitochondria, soluble intracellular binding proteins, mineralized Pb (i.e., hydroxyapatite) and inclusion bodies ([Long et al., 1990](#); [Pounds and Rosen, 1986](#); [Rosen, 1983](#)). Approximately 70-80% of Pb taken up into isolated primary cultures of osteoblasts or osteocytes is associated with mitochondria and mineralized Pb ([Pounds et al., 1991](#)).

Pb accumulates in bone regions having the most active calcification at the time of exposure. Pb accumulation is thought to occur predominantly in trabecular bone during

childhood and in both cortical and trabecular bone in adulthood ([Aufderheide and Wittmers, 1992](#)). Early Pb uptake in children is greater in trabecular bone due to its larger surface area and higher metabolic rate. With continued exposure, Pb concentrations in bone may increase with age throughout the lifetime beginning in childhood, indicative of a relatively slow turnover of Pb in adult bone ([Park et al., 2009b](#); [Barry and Connolly, 1981](#); [Barry, 1975](#); [Gross et al., 1975](#); [Schroeder and Tipton, 1968](#)). The cortical and trabecular bones have distinct rates of turnover and Pb release. For example, tibia has a turnover rate of about 2% per year whereas trabecular bone has a turnover rate of more than 8% per year in adults ([Rabinowitz, 1991](#)).

A high bone formation rate in early childhood results in the rapid uptake of circulating Pb into mineralizing bone; however, bone Pb is also recycled to other tissue compartments or excreted in accordance with a high bone resorption rate ([O'Flaherty, 1995](#)). Thus, most of the Pb acquired early in life is not permanently fixed in the bone (60-65%) ([O'Flaherty, 1995](#); [Leggett, 1993](#); [ICRP, 1973](#)). However, some Pb accumulated in bone does persist into later life. McNeill et al. ([2000](#)) compared tibia Pb levels and cumulative blood Pb indices in a population of 19- to 29-year-olds who had been highly exposed to Pb in childhood from the Bunker Hill, Idaho smelter; they concluded that Pb from exposure in early childhood had persisted in the bone matrix until adulthood.

Additional discussion of the Pb in bone and its mobilization are provided in other sections of this chapter. Maternal mobilization of Pb from the bone to the fetus is discussed in [Section 3.2.2.4](#). The relationship between Pb in blood and bone is discussed in [Section 3.3.5](#).

3.2.2.3 Soft Tissues

Most of the Pb in soft tissue is in liver and kidney ([Gerhardsson et al., 1995](#); [Oldereid et al., 1993](#); [Gerhardsson et al., 1986](#); [Barry, 1981, 1975](#); [Gross et al., 1975](#)). Presumably, the Pb in these soft tissues (i.e., 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](#)). The Pb-binding proteins in rat are cleavage products of $\alpha_2\mu$ globulin, a member of the protein superfamily known as retinol-binding proteins that are generally observed only in male rats ([Fowler and DuVal, 1991](#)). Other high-affinity Pb-binding proteins ($K_d \sim 14$ nM) have been isolated in human kidney, two of which have been identified as a 5 kDa peptide, thymosin 4 and a 9 kDa peptide, acyl-CoA binding protein ([Smith et al., 1998](#)). Pb also binds to metallothionein, but does not appear to be a significant inducer of the protein in comparison with the inducers Cd and Zn ([Waalkes and Klaassen, 1985](#); [Eaton et al., 1980](#)).

The liver and kidneys rapidly accumulate systemic Pb ($t_{1/2}$ = 0.21 and 0.41 hours, respectively), which amounts to 10-15% and 15-20% of intravenously injected Pb, respectively ([Leggett, 1993](#)). A linear relationship in dose-tissue Pb concentrations for kidney and liver has been demonstrated in swine, dogs, and rats ([Smith et al., 2008](#); [Casteel et al., 2006](#); [Casteel et al., 1997](#); [Azar et al., 1973](#)). 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 ([Treble and Thompson, 1997](#); [Barry, 1975](#)), reflecting a faster turnover of Pb in soft tissue relative to bone.

3.2.2.4 Fetus

Evidence for maternal-to-fetal transfer of Pb in humans is derived from cord blood Pb to maternal blood Pb ratios (i.e., cord blood Pb concentration divided by mother's blood Pb concentration). Group mean ratios range from about 0.7 to 1.0 at the time of delivery for mean maternal blood Pb levels ranging from 1.7 to 8.6 µg/dL ([Amaral et al., 2010](#); [Kordas et al., 2009](#); [Patel and Prabhu, 2009](#); [Carbone et al., 1998](#); [Goyer, 1990](#); [Graziano et al., 1990](#)). The relationship for individual mother-child pairs is variable, but well correlated (Pearson r = 0.79); in a predominantly young, low income, urban population (N = 159), factors associated with higher cord compared to maternal blood Pb level included maternal elevated blood pressure and alcohol consumption, while factors associated with relatively lower ratios of cord blood Pb to maternal blood Pb included maternal increased hemoglobin levels and sickle cell trait ([Harville et al., 2005](#)). Calcium intake and physical activity were not associated with differences between cord Pb and maternal blood Pb. Consistent with other studies, the ratio of mean cord blood Pb (1.64 µg/dL) to mean maternal blood Pb (1.93 µg/dL) was 0.85. The similarity of isotopic ratios in maternal blood and in blood and urine of newly-born infants provides further evidence for placental transfer of Pb to the fetus ([Gulson et al., 1999](#)).

Transplacental transfer of Pb may be facilitated by an increase in the plasma/blood Pb concentration ratio during pregnancy ([Montenegro et al., 2008](#); [Lamadrid-Figueroa et al., 2006](#)). Maternal-to-fetal transfer of Pb appears to be related partly to the mobilization of Pb from the maternal skeleton. Evidence for transfer of maternal bone Pb to the fetus has been provided by stable Pb isotope studies in cynomolgus monkeys exposed during pregnancy. Approximately 7-39% of the maternal Pb burden transferred to the fetus was derived from the maternal skeleton, with the remainder derived from contemporaneous exposure ([O'Flaherty, 1998](#); [Franklin et al., 1997](#)). The upper value in the range (39%) represented the one monkey with historical Pb exposure, but received only small amounts of environmental Pb exposure during pregnancy; for the monkeys that received high

doses of Pb during pregnancy, the range was lower (7-25%) ([O'Flaherty, 1998](#); [Franklin et al., 1997](#)).

3.2.2.5 Organic Pb

Information on the distribution of Pb in humans following exposures to organic Pb is extremely limited. However, as reported in the 2006 Pb AQCD ([U.S. EPA, 2006c](#)), the available evidence demonstrates near complete absorption following inhalation of tetraalkyl Pb vapor and subsequent transformation to trialkyl Pb metabolites. One hour following brief inhalation exposures to ^{203}Pb tetraethyl or tetramethyl Pb (1 mg/m^3), ~50% of the ^{203}Pb body burden was associated with liver and 5% with kidney; the remaining ^{203}Pb was widely distributed throughout the body ([Heard et al., 1979](#)). The kinetics of ^{203}Pb in blood showed an initial declining phase during the first 4 hours (tetramethyl Pb) or 10 hours (tetraethyl Pb) after the exposure, followed by a reappearance of radioactivity back into the blood after ~20 hours. 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](#)).

Alkyl Pb compounds undergo oxidative dealkylation catalyzed by cytochrome P450 in liver and, possibly, in other tissues. Trialkyl Pb metabolites have been found in the liver, kidney, and brain following exposure to the tetraalkyl compounds in workers ([Bolanoska et al., 1967](#)); these metabolites have also been detected in brain tissue of nonoccupational subjects ([Nielsen et al., 1978](#)).

3.2.3 Elimination

The rapid-phase (30-40 days) of Pb excretion amounts to 50-60% of the absorbed fraction ([Chamberlain et al., 1978](#); [Rabinowitz et al., 1976](#); [Kehoe, 1961a, b, c](#)).

Absorbed Pb is excreted primarily in urine and feces, with sweat, saliva, hair, nails, and breast milk being minor routes of excretion ([Kehoe, 1987](#); [Chamberlain et al., 1978](#); [Rabinowitz et al., 1976](#); [Griffin et al., 1975](#); [Hursh et al., 1969](#); [Hursh and Suomela, 1968](#)).

Approximately 30% of intravenously injected Pb in humans (40-50% in beagles and baboons) is excreted via urine and feces during the first 20 days following administration ([Leggett, 1993](#)). The kinetics of urinary excretion following a single dose of Pb is similar to that of blood ([Chamberlain et al., 1978](#)), likely due to the fact that Pb in urine derives

largely from Pb in plasma. Evidence for this is the observation that urinary Pb excretion is strongly correlated with the rate of glomerular filtration of Pb ([Araki et al., 1986](#)) and plasma Pb concentration ([Rentschler et al., 2012](#); [Bergdahl et al., 1997b](#)) (i.e., glomerular filtration rate $\times$ plasma Pb concentration), and both relationships are linear. While the relationship between urinary Pb excretion and plasma Pb concentration is linear, the plasma Pb relationship to blood Pb concentration is curvilinear (as described in [Section 3.2.2.1](#) and demonstrated in [Figure 3-6](#)). This relationship contributes to an increase in the renal clearance of Pb from blood with increasing blood Pb concentrations ([Chamberlain, 1983](#)). Similarly, a linear relationship between plasma Pb concentration and urinary excretion rate predicts a linear relationship between Pb intake (at constant absorption fraction) and urinary Pb excretion rate, whereas the relationship with blood Pb concentration would be expected to be curvilinear ([Section 3.3.6](#)).

Estimates of urinary filtration of Pb from plasma range from 13-22 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](#)), which corresponds to half-time for transfer of Pb from plasma to urine of 0.1-0.16 days for a 70-kg adult who has a plasma volume of $\sim$ 3 L. The rate of urinary excretion of Pb was less than the rate of glomerular filtration of ultrafilterable Pb, suggesting that urinary Pb is the result of incomplete renal tubular reabsorption of Pb in the glomerular filtrate ([Araki et al., 1986](#)); although, net tubular secretion of Pb has been demonstrated in animals ([Victory et al., 1979](#); [Vander et al., 1977](#)). On the other hand, estimates of blood-to-urine clearance range from 0.03-0.3 L/day with a mean of 0.18 L/day ([Diamond, 1992](#); [Araki et al., 1990](#); [Berger et al., 1990](#); [Koster et al., 1989](#); [Manton and Malloy, 1983](#); [Ryu et al., 1983](#); [Chamberlain et al., 1978](#); [Rabinowitz et al., 1973](#)), consistent with a plasma Pb to blood Pb concentration ratio of $\sim$ 0.005–0.01 L/day ([Klotzback et al., 2003](#)). Based on the above differences, urinary excretion of Pb can be expected to reflect the concentration of Pb in plasma and variables that affect delivery of Pb from plasma to urine (e.g., glomerular filtration and other transfer processes in the kidney).

The value for fecal:urinary excretion ratio ($\sim$ 0.5) was observed during days 2-14 following intravenous injection of Pb in humans ([Chamberlain et al., 1978](#); [Booker et al., 1969](#); [Hursh et al., 1969](#)). This ratio is slightly higher (0.7-0.8) with inhalation of submicron Pb-bearing PM due to ciliary clearance and subsequent ingestion. The transfer of Pb from blood plasma to the small intestine by biliary secretion in the liver is rapid (adult $t_{1/2}$ = 10 days), and accounts for 70% of the total plasma clearance ([O'Flaherty, 1995](#)).

Organic Pb

Pb absorbed after inhalation of tetraethyl and tetramethyl Pb is excreted in exhaled air, urine, and feces ([Heard et al., 1979](#)). 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 exposed to tetraethyl Pb showed that tetraethyl Pb is excreted in the urine as diethyl Pb, ethyl Pb, and inorganic Pb ([Vural and Duydu, 1995](#); [Zhang et al., 1994](#); [Turlakiewicz and Chmielnicka, 1985](#)).

3.3 Pb Biomarkers

This section describes the biological measurements of Pb and their interpretation as indicators of exposure or body burden.

For any health endpoint of interest, the most useful biomarker of exposure is one that provides information about the Pb dose at the critical target organ and, moreover, reflects the exposure averaging time that is appropriate to the underlying pathogenetic processes (e.g., instantaneous, cumulative over lifetime, or cumulative over a circumscribed age range). In recent studies of Pb and health, the exposure biomarkers most frequently used are Pb in blood and bone. For outcomes other than those relating to hematopoiesis and bone health, these biomarkers provide information about Pb dose that is some distance from the target organ. For example, given that the central nervous system is considered the critical target organ for childhood Pb toxicity, it would be most helpful to be able to measure, in vivo, the Pb concentrations at the cellular site(s) of action in the brain. However, because such measurements are not currently feasible, investigators must rely on measurements of Pb in the more readily accessible but peripheral tissues. The relationship between brain Pb and Pb in each of these surrogate tissues is still poorly understood, although the pharmacokinetics clearly differs among these compartments.

As an exposure biomarker, blood Pb concentration has other limitations. Only about 1% of an individual's total body Pb burden resides in blood. Furthermore, blood consists of several subcompartments. More than 90% of Pb in whole blood is bound to red cell proteins such as ALAD, with the balance in plasma. From a toxicological perspective, the unbound fraction is likely to be the most important subcompartment of blood Pb because it distributes into soft tissues. The concentration of Pb in plasma is much lower than in whole blood (<1%). The greater relative abundance of Pb in whole blood makes its measurement much easier (and more affordable) than measurement of Pb in plasma. The use of whole blood Pb as a surrogate for plasma Pb could be justified if the ratio of whole blood Pb to plasma Pb were well characterized, but this is not so. At least some studies suggest that it varies several-fold among individuals with the same blood Pb level.

Moreover, binding Pb in red blood cells is limited, so the ratio of blood Pb to plasma Pb would be expected to be nonlinear. Thus, interpreting whole blood Pb level as a proxy for plasma Pb level, which, itself, is a proxy for brain Pb level, will result in some exposure misclassification.

Another limitation of blood Pb as an exposure biomarker is that the kinetics of Pb in blood is relatively fast compared to the kinetics of Pb in bone, and therefore, of the whole body burden. Thus, a high blood Pb concentration measured at any given time does not necessarily indicate a high body Pb burden. Similarly, individuals who have the same blood Pb level will not necessarily have similar body burdens or exposure histories. The rate at which blood Pb changes with time/age depends on exposure history due to re-equilibration of Pb stored in the various body pools.

The development of X-ray-fluorescence (XRF) methods for measuring Pb in mineralized tissues offers another approach for characterization and reconstruction of exposure history. Such tissues are long-term Pb storage sites, with a half-life measured in decades and contain ~90% of the total body Pb burden in adults and 70% in children. Thus, bone Pb reflects a long exposure averaging time.

Mechanistic models are used throughout the section as a means to describe basic concepts that derive from the wealth of information on Pb toxicokinetics. Although predictions from models are inherently uncertain, models can serve to illustrate expected interrelationships between Pb intake and tissue distribution that are important in interpreting human clinical and epidemiologic studies. Thus, models serve as the only means available for synthesizing the extensive, but incomplete, knowledge of Pb biokinetics into a holistic representation of Pb biokinetics. Furthermore, models can also be used to make predictions about biokinetics relationships that have not been thoroughly evaluated in experiments or epidemiologic studies. In this way, models can serve as heuristic tools for shaping data collection to improve understanding of Pb biokinetics.

Mechanistic toxicokinetics models can make predictions about hypothetical populations and exposure scenarios. When a model is run as a single simulation, the output represents average outcomes from what is in reality a distribution of possible outcomes that would be expected in the population (or in any single individual) where intra-individual and inter-individual variability in exposure and toxicokinetics exist. More realistic predictions for the population can be developed by running a series of model simulations in which ranges (i.e., distributions) of parameter values are considered that may better represent the population of interest. In this section, only single simulations are used to demonstrate relationships between various biomarkers (e.g., blood Pb and bone Pb) that would apply to a population having “typical” or “average” exposure and toxicokinetics. These single

simulations are used for illustrative purposes to describe general concepts and patterns. Variability would be expected in real populations.

Numerous mechanistic models of Pb biokinetics in humans have been proposed, and these are described in the 2006 Pb AQCD ([U.S. EPA, 2006b](#)) and in the supporting literature cited in that report. In this section, for simplicity and for internal consistency, discussion is limited to predictions from a single model, the ICRP Pb biokinetics model ([Pounds and Leggett, 1998](#); [ICRP, 1994](#); [Leggett, 1993](#)). The ICRP model consists of a systemic biokinetics model ([Leggett, 1993](#)) and a human respiratory tract model ([ICRP, 1994](#)). The Leggett model simulates age-dependent kinetics of tissue distribution and excretion of Pb ingestion and inhalation intakes. This model was originally developed for the purpose of supporting radiation dosimetry predictions and it has been used to develop cancer risk coefficients for internal radiation exposures to Pb and other alkaline earth elements that have biokinetics similar to those of calcium ([ICRP, 1993](#)). Although the ICRP model has not been validated by U.S. EPA as a regulatory model for Pb risk assessment, it has been applied in Pb risk assessment ([Abrahams et al., 2006](#); [Lorenzana et al., 2005](#); [Khoury and Diamond, 2003](#)). Portions of the model have been incorporated into an AALM that is being developed by EPA ([2005a](#)). In addition to the above considerations regarding previous applications of the ICRP model, the model was selected for use in the ISA because it has several useful features for predicting exposure-body burden relationships. The model simulates blood Pb and tissue Pb concentration dynamics associated with the uptake and elimination phases of exposures of ≥ 1 day in duration; and it simulates age-dependent and particle size-dependent deposition and clearance of inhaled Pb in the respiratory tract. These types of simulations can only be approximated with the U.S. EPA IEUBK Model for Pb in children because it simulates exposures in time steps of 1 year (i.e., age-year average exposures); lumps the simulation of deposition, mechanical clearance, and absorption of inhaled Pb into a single absorption term representing the combined processes of gastrointestinal and respiratory tract absorption of inhaled Pb; simulates steady state blood Pb concentrations and does not allow access to the underlying simulations of tissue Pb concentrations which serve as intermediate variables in the model for predicting steady state blood Pb concentrations. Other models have been developed that allow simulations of tissue Pb concentrations (e.g., [O'Flaherty, 1995](#); [Leggett, 1993](#)) and comparisons of these models have been previously described ([Maddaloni et al., 2005](#)).

Pb biokinetics in adolescents is poorly characterized by all existing Pb biokinetics models. Individuals undergo rapid changes in sexual development, growth, food and water intake, bone growth and turnover, behavior, etc. during adolescence. There is a paucity of experimental measurements of Pb biomarkers during this time developmental window. The individual biological and kinetic parameters for adolescents are largely interpolated rather than based on solid experimental and toxicological measurements. These deficiencies limit the validity of model predictions in this age group.

3.3.1 Bone-Pb Measurements

For Pb measurements in bone, the most commonly examined bones are the tibia, calcaneus, patella, and finger bone. For cortical bone, the midpoint of the tibia is measured. For trabecular bone, both the patella and calcaneus are measured. The tibia consists of more than 95% cortical bone, the calcaneus and patella comprise more than 95% trabecular bone, and finger bone is a mixed cortical and trabecular bone although the second phalanx is dominantly cortical. Recent studies favor measurement of the patella for estimating trabecular bone Pb, because it has more bone mass and may afford better measurement precision than the calcaneus.

Bone Pb measurements are typically expressed in units of $\mu\text{g Pb per g bone mineral}$. This convention may potentially introduce variability into the bone Pb measurements related to variation in bone density. Typically, potential associations between bone density and bone Pb concentration are not evaluated in epidemiologic studies ([Theppeang et al., 2008a](#); [Hu et al., 2007a](#)). 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.

Methods of direct analysis of bone tissue samples include flame atomic absorption spectrometry (AAS), anode stripping voltammetry (ASV), inductively coupled plasma atomic emission spectroscopy (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS), laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), thermal ionization mass spectrometry (TIMS), synchrotron radiation induced X-ray emission (SRIXE), particle induced X-ray emission (PIXE), and X-ray fluorescence (XRF). Non-invasive, in vivo measurements of bone Pb is achieved with XRF. The upsurge in popularity of the XRF method has paralleled a decline in the use of the other methods. More information on the precision, accuracy, and variability in bone Pb measurements can be found in the 2006 Pb AQCD ([U.S. EPA, 2006b](#)).

Two main approaches for XRF measurements have been used to measure Pb concentrations in bone, the K-shell and L-shell methods. The K-shell method is the most widely used, as there have been relatively few developments in L-shell devices since the early 1990s. However, Nie et al. ([2011a](#)) recently reported on the use of a new portable L-shell device for human in vivo Pb measurements. Advances in L-shell device technology resulted in much higher sensitivity than previous L-shell devices. The new L-shell device showed sensitivity similar to that of K-shell methods (detection limit was approximately 8 µg/g bone mineral with 2 mm of soft tissue overlay targeted bone) and a high correlation with results obtained from K-shell methods (intraclass correlation = 0.65). Behinaen et al. ([2011](#)) described application of a 4-detector system (“*clover leaf array*”) for the K-shell method that provided higher precision and lower minimum detection limits (MDL) for tibia and calcaneus Pb measurements (3.25 and 4.78 µg/g bone mineral, respectively) compared to measurements made with single detectors (8-12 µg/g and 14-15 µg/g, respectively).

Since 1986, several investigators have reported refinements to hardware and software to improve the precision and accuracy of XRF measurements and there have been a number of investigations into the precision, accuracy and variability in XRF measurements [e.g., ([Todd et al., 2002](#); [Todd et al., 2001](#); [Aro et al., 2000](#); [Todd et al., 2000](#))]. Todd et al. ([2000](#)) provided a detailed discussion of factors that influence the variability and measurement uncertainty, including repositioning, sample measurement duration, overlying tissue, operator expertise, detector resolution, and changes to measurement process over time. Some of these aspects were also discussed by Hu et al. ([1995](#)). From their cadaver and in vivo measurements, Todd et al. ([2000](#)) concluded that the uncertainty in an individual measurement was an underestimate of the standard deviation of replicate measurements, suggesting a methodological deficiency probably shared by most current ¹⁰⁹Cd-based K-shell XRF Pb measurement systems. In examining the reproducibility of the bone Pb measurements over a 4½ month period, Todd et al. ([2000](#)) also found the average difference between the XRF results from short term and longer term measurements was 1.2 µg/g, indicating only a small amount of variability in the XRF results over a sustained period of time.

In the epidemiologic literature, XRF bone Pb data have typically been reported in two ways: one that involves a methodological approach to assessing the minimum detection limit and the other termed an epidemiologic approach by Rosen and Pounds ([1998](#)). In the former approach, a minimum detection limit is defined using various methods, including two or three times the square root of the background counts; one, two, or three times the standard deviation (SD) of the background; or two times the observed median error. This approach relies upon the minimum detection limit to define a quantitative estimate that is of sufficient precision to be included in the statistical analysis, as

demonstrated by Bellinger et al. ([1994a](#)), Gerhardsson et al. ([1993](#)), and Christoffersson et al. ([1986](#)).

With the epidemiologic approach, all values are used (including negative values) to determine the minimum detection limit of an instrument that results in extremely low detection limits. Rosen and Pounds ([1998](#)) noted that this approach yields population bone Pb averages that were artificially low. However, not including values that are negative or below the detection limit, or assigning these values a fixed number is also of concern. Using the epidemiologic approach of retaining all point estimates of measured bone Pb concentrations provided the least amount of bias and the greatest efficiency in comparing the mean or median levels of bone Pb of different populations ([Kim et al., 1995](#)).

3.3.2 Blood-Pb Measurements

Analytical methods for measuring Pb in blood include AAS, graphite furnace atomic absorption spectrometry (GFAAS), ASV, ICP-AES, and ICP-MS. GFAAS and ASV are generally considered to be the methods of choice ([Flegal and Smith, 1995](#)). Limits of detection for Pb using AAS are on the order of 5-10 µg/dL for flame AAS measurements and approximately 0.1 µg/dL for flameless AAS measurements ([Flegal and Smith, 1995](#); [NIOSH, 1994](#)). A detection limit of 0.005 µg/dL has been achieved for Pb in blood samples analyzed by GFAAS.

For measurement of Pb in plasma, ICP-MS provides sufficient sensitivity ([Schutz et al., 1996](#)). While the technique has been applied to assessing Pb exposures in adults, ICP-MS has not received widespread use in epidemiologic studies. Maternal blood and cord blood samples have been utilized to assess blood Pb concentrations of neonates (e.g., [Amaral et al., 2010](#); [Carbone et al., 1998](#)).

The primary binding ligand for Pb in RBC, ALAD, is encoded by a single gene in humans that is polymorphic in two alleles (ALAD1 and ALAD2) ([Scinicariello et al., 2007](#)). Since the ALAD1 and ALAD 2 alleles can be co-dominantly expressed, 3 different genotypes (ALAD 1-1, ALAD 1-2, and ALAD 2-2) are possible. The ALAD 1-1 genotype is the most common. Scinicariello et al. ([2010](#)) tested genotypes in civilian, non-institutionalized U.S. individuals that participated as part of NHANES III from 1988–1994 and found that 15.6% of non-Hispanic whites, 2.6% non-Hispanic blacks, and 8.8% Mexican Americans carried the ALAD2 allele.

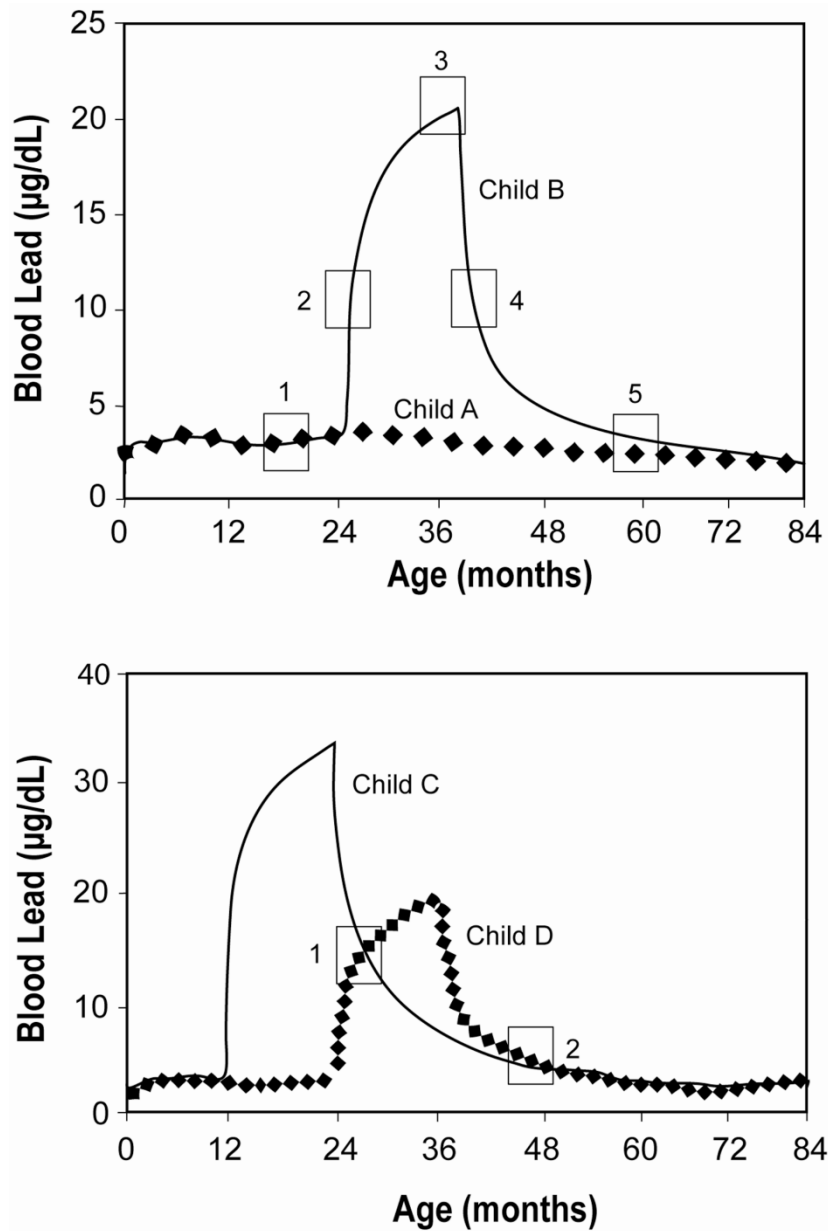
The 2006 Pb AQCD ([U.S. EPA, 2006c](#)) reported that many studies have shown that, with similar exposures to Pb, individuals with the ALAD-2 allele have higher blood Pb levels

than those without ([Kim et al., 2004](#); [Pérez-Bravo et al., 2004](#); [Bergdahl et al., 1997b](#); [Smith et al., 1995a](#); [Wetmur, 1994](#); [Wetmur et al., 1991b](#); [Astrin et al., 1987](#)). More recent meta analyses provide further support for ALAD2 carriers having higher blood Pb levels than ALAD1-1 homozygotes ([Scinicariello et al., 2007](#); [Zhao et al., 2007](#)). The mechanism for this association may be higher Pb binding affinity of ALAD2. Although, this interpretation would be consistent with the structural differences that result in greater electronegativity of ALAD1 compared to ALAD2 ([Wetmur, 1994](#); [Wetmur et al., 1991a](#)), measurements of Pb binding affinity to ALAD1 and ALAD2 (i.e., Pb^{2+} displacement of Zn^{2+} binding to recombinant ALAD1 and ALAD2) have not revealed differences in Pb binding affinity ([Jaffe et al., 2000](#)). In a meta-analysis of 24 studies, Scinicariello et al. ([2007](#)), observed the greatest differences for ALAD2 compared to ALAD1 in highly exposed adults with little difference among environmentally-exposed adults; large differences were also observed for children at low exposures. However, there are few studies that evaluated children and the largest study contributing to the meta analysis may have been influenced by selection bias ([Scinicariello et al., 2007](#)). Individual studies find similar results in occupationally-exposed adults, with blood Pb levels being higher in individuals with ALAD2 alleles ([Miyaki et al., 2009](#); [Shaik and Jamil, 2009](#)). A subsequent meta analysis of adult data from NHANES III did not find any differences in blood Pb level between all carriers of either the ALAD 1-1 or ALAD 1-2/2-2 allele ([Scinicariello et al., 2010](#)). Other studies provide further support for no blood Pb differences among ALAD1 and ALAD2 carriers ([Sobin et al., 2009](#); [Rabstein et al., 2008](#); [Montenegro et al., 2006](#); [Wananukul et al., 2006](#)) or lower blood Pb levels for individuals with ALAD 1-2/2-2 ([Krieg et al., 2009](#); [Chia et al., 2006](#)). The contribution of ALAD alleles to blood Pb levels is inconclusive and the underlying causes of discrepancies among studies remain to be elucidated.

Genetic polymorphism in the gene that encodes for peptide transporter 2 (PEPT2) has been associated with variability in blood Pb concentrations in children ([Sobin et al., 2009](#)). PEPT2 expression in the brain and renal proximal tubule has been associated with transport of di- and tri-peptides and may function in the transport of δ -ALA into brain and renal tubular reabsorption of peptides. The PRPT2*2 polymorphism was associated with increased blood Pb concentrations in a sample of 116 children of Mexican-American/Hispanic heritage (age 4-12 years, mean blood Pb concentration 3-6 $\mu\text{g}/\text{dL}$).

Analyses of serial blood Pb concentrations measured in longitudinal epidemiologic studies found relatively strong correlations (e.g., $r = 0.5\text{-}0.8$) among each child's individual blood Pb concentrations measured after 6-12 months of age ([Schnaas et al., 2000](#); [Dietrich et al., 1993a](#); [McMichael et al., 1988](#); [Otto et al., 1985](#); [Rabinowitz et al., 1984](#)). These observations suggest that, in general, exposure characteristics of an individual child (e.g., exposure levels and/or exposure behaviors) tend to be relatively

constant across age. However, a single blood Pb measurement may not distinguish between a history of long-term lower-level Pb exposure from a history that includes higher acute exposures ([Mushak, 1998](#)). This concept is illustrated in [Figure 3-7](#). Two hypothetical children are simulated. Child A has a relatively constant Pb intake from birth, whereas Child B has the same Pb intake as Child A for the first two years of life, then a 1-year elevated intake beginning at age 24 months ([Figure 3-7](#), upper panel) that returns to the same intake as Child A at 36 months. The absorption fraction is assumed to be the same for both children. Blood Pb samples 1 and 5 for Child A and B, or 2 and 4 for Child B, will yield similar blood Pb concentrations (~ 3 or $10 \mu\text{g/dL}$, respectively), yet the exposure contexts for these samples are very different. Two samples (e.g., 1 and 2, or 4 and 5), at a minimum, are needed to ascertain if the blood Pb concentration is changing over time. The rate of change can provide information about the magnitude of change in exposure, but not necessarily about the time history of the change ([Figure 3-7](#), lower panel). Time-integrated measurements of Pb concentration may provide a means for accounting for some of these factors and, thereby, provide a better measure of long-term Pb exposure.



Note: Child A and Child B had a constant basal Pb intake (10 µg/day) from birth; in addition to the basal intake of 10 µg/day, Child B experienced an elevated intake of 5.5 µg/kg per day (i.e., an average of 88 µg/day) for 1 year beginning at 24 months of age (upper panel). Blood Pb measurements 1 and 5 for Child A and B, or 2 and 4 for Child B, will yield similar blood Pb concentrations (~3 or 10 µg/dL, respectively), yet the exposure scenarios for these samples are very different. As shown in the example of Child C and Child D, two blood Pb measurements can provide information about the magnitude of change in exposure, but not necessarily the temporal history of the change (lower panel). Child C and D had a constant basal Pb intake (10 µg/day) from birth. In addition to the basal intake of 10 µg/day, Child C experienced an elevated intake of 13 µg/kg per day (i.e., an average of 160 µg/day) for 1 year beginning at 12 months of age, whereas, Child D experienced the same exposure as Child B. Simulation based on ICRP Pb biokinetics model ([Leggett, 1993](#)).

Figure 3-7 **Simulation of temporal relationships between Pb exposure and blood Pb concentration in children.**

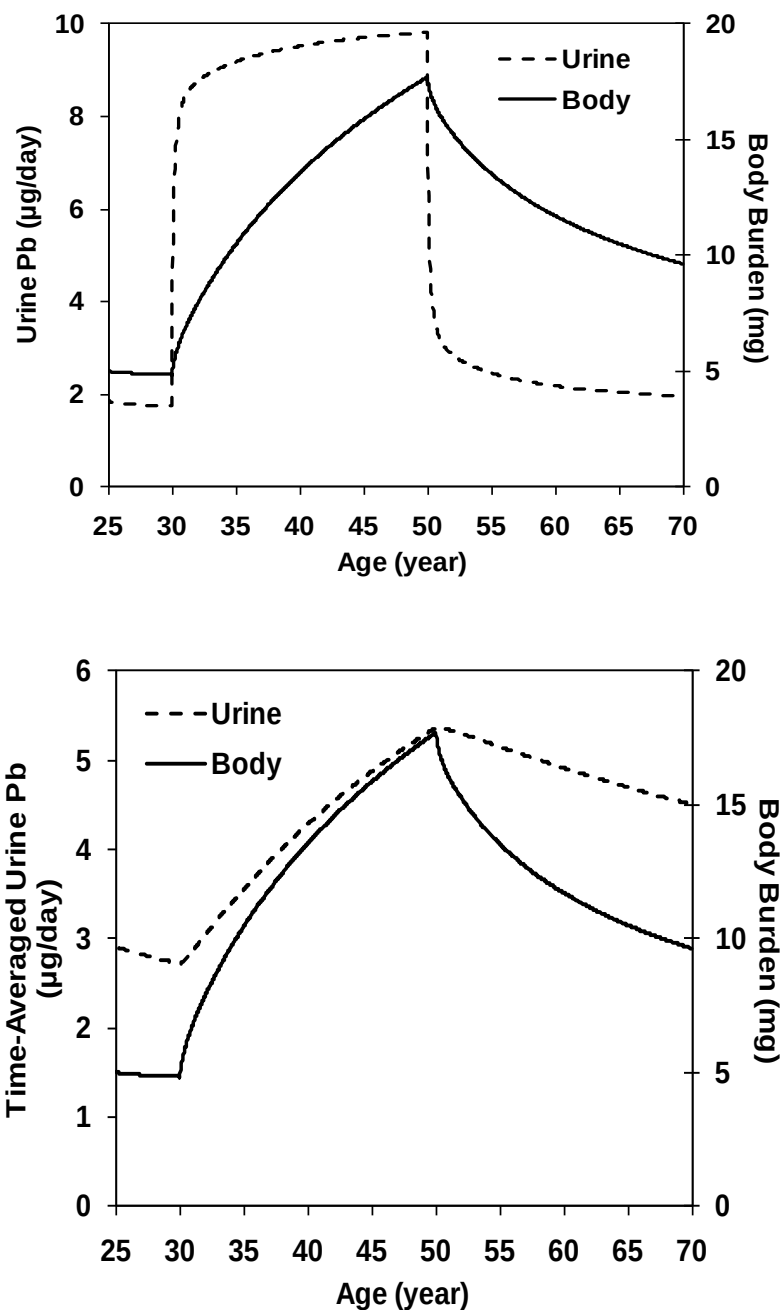
3.3.3 Urine-Pb Measurements

Standard methods that have been reported for urine Pb analysis are, in general, the same as those analyses noted for determination of Pb in blood. Reported detection limits are ~50 µg/L for AAS, 5-10 µg/L for ICP AES, and 4 µg/L for ASV for urine Pb analyses.

The concentration of Pb in urine is a function of the urinary Pb excretion ([Section 3.2.3](#)) and the urine flow rate. Urine flow rate requires collection of a timed urine sample, which is often problematic in epidemiologic studies. Collection of untimed (“spot”) urine samples, a common alternative to timed samples, requires adjustment of the Pb measurement in urine to account for variation in urine flow ([Diamond, 1988](#)). Several approaches to this adjustment have been explored, including adjusting the measured urine Pb concentration by the urine creatinine concentration, urine osmolality, or specific gravity ([Fukui et al., 1999](#); [Araki et al., 1990](#)). Urine flow rate can vary by a factor or more than 10, depending on the state of hydration and other factors that affect glomerular filtration rate and renal tubular reabsorption of the glomerular filtrate. All of these factors can be affected by Pb exposure at levels that produce nephrotoxicity (i.e., decreased glomerular filtration rate, impaired renal tubular transport function). Therefore, urine Pb concentration measurements provide little reliable information about exposure (or Pb body burden), unless they can be adjusted to account for unmeasured variability in urine flow rate ([Araki et al., 1990](#)).

Urinary Pb concentration reflects, mainly, the concentration of Pb in the blood. As such, urinary concentrations reflect both recent and past exposures to Pb (see [Section 3.3.5](#)). A single urinary Pb measurement cannot distinguish between a long-term low level of exposure or a higher acute exposure. Urinary Pb measurements would be expected to correlate with concurrent blood Pb (see [Section 3.3.6](#) for additional discussion of the relationship between blood and urine Pb). Chiang et al. ([2008](#)) reported a significant, but relatively weak correlation between urinary Pb levels (µg/dg creatinine) and individual Pb intakes (µg/day) estimated in a group of 10- to 12-year-old children (β : 0.053, $R = 0.320$, $p = 0.02$, $N = 57$). A contributing factor to the relatively weak correlation may have been the temporal displacement between the urine sampling and measurements used to estimate intake, which may have been as long as 6 months for some children.

Thus, a single urine Pb measurement, or a series of measurements taken over short-time span, is likely a relatively poor index of Pb body burden for the same reasons that blood Pb is not a good indicator of body burden. On the other hand, long-term average measurements of urinary Pb can be expected to be a better index of body burden ([Figure 3-8](#)).



Note: A change in Pb uptake results in a relatively rapid change in urinary excretion of Pb, to a new quasi-steady state, and a relatively small change in body burden (upper panel). Baseline ingestion was 20 $\mu\text{g/day}$ from age 0 to 30 yrs, then intake increased to 120 $\mu\text{g/day}$ from age 30 to 50 with a subsequent decrease in intake to the baseline of 20 $\mu\text{g/day}$ at age 50. The long-term average urinary Pb excretion more closely tracks the pattern of change in body burden (lower panel). Simulation based on ICRP Pb biokinetics model ([Leggett, 1993](#)).

Figure 3-8 Simulation of relationship between urinary Pb excretion and body burden in adults.

3.3.4 Pb in Other Biomarkers

There was extensive discussion in the 2006 Pb AQCD ([U.S. EPA, 2006c](#)) regarding the utility of other Pb biomarkers as indicators of exposure or body burden. Due to the fact that most epidemiologic studies continue to use blood Pb or bone Pb, and other potential biomarkers (i.e., teeth, hair, and saliva) have not been established to the same extent as blood or bone Pb, only summaries are provided below.

3.3.4.1 Teeth

Tooth Pb is a minor contributor to the total body burden of Pb. As teeth accumulate Pb, tooth Pb levels are generally considered an estimate of cumulative Pb exposure. The tooth Pb-blood Pb relationship is more complex than the bone Pb-blood Pb relationship because of differences in tooth type, location, and analytical method. Although mobilization of Pb from bone appears well established, this is not the case for Pb in teeth. Conventional wisdom has Pb fixed once it enters the tooth. Although that may be the case for the bulk of enamel, it is not true for the surface of the enamel and dentine ([Gulson et al., 1997](#); [Rabinowitz et al., 1993](#)). Limited studies have demonstrated moderate-to-high correlations between tooth Pb levels and blood Pb levels ([Rabinowitz, 1995](#); [Rabinowitz et al., 1989](#)).

Teeth are composed of several tissues formed pre- and postnatal. Therefore, if a child's Pb exposure during the years of tooth formation varied widely, different amounts of Pb would be deposited at different rates ([Rabinowitz et al., 1993](#)). This difference may allow investigators to elucidate the history of Pb exposure in a child. Robbins et al. ([2010](#)) found a significant association between environmental Pb measures that correlated with leaded gasoline use and tooth enamel Pb in permanent teeth. Costa de Almeida et al. ([2007](#)) discerned differences between tooth enamel Pb concentration in biopsy samples from children who lived in areas having higher or lower levels of Pb contamination. Gulson and Wilson ([1994](#)) advocated the use of sections of enamel and dentine to obtain additional information compared with analysis of the whole tooth (e.g., ([Tvinnereim et al., 1997](#); [Fosse et al., 1995](#))). For example, deciduous tooth Pb in the enamel provides information about in utero exposure whereas that in dentine from the same tooth provides information about postnatal exposure until the tooth exfoliates at about 6-7 years of age.

3.3.4.2 Hair

The 2006 Pb AQCD ([U.S. EPA, 2006c](#)) discussed applications of hair Pb measurements for assessing Pb body burden or exposure and noted methodological limitations (e.g., external contamination) and lack of a strong empirical basis for relating hair Pb levels to body burden or exposure. No new methodological or conceptual advances regarding hair Pb measurements have occurred since 2006, and widespread application of hair Pb measurements in epidemiologic studies has not occurred.

Pb is incorporated into human hair and hair roots ([Bos et al., 1985](#); [Rabinowitz et al., 1976](#)) and has been explored as a noninvasive approach for estimating Pb body burden ([Wilhelm et al., 2002](#); [Gerhardsson et al., 1995](#); [Wilhelm et al., 1989](#)). Hair Pb measurements are subject to error from contamination of the surface with environmental Pb and contaminants in artificial hair treatments (i.e., dyeing, bleaching, permanents) and are a relatively poor predictor of blood Pb concentrations, particularly at blood Pb levels less than 10-12 µg/dL ([Rodrigues et al., 2008](#); [Campbell and Toribara, 2001](#); [Esteban et al., 1999](#); [Drasch et al., 1997](#)). Temporal relationships between Pb exposure and hair Pb levels, and kinetics of deposition and retention of Pb in hair have not been evaluated. Although hair Pb measurements have been used in some epidemiologic studies ([Shah et al., 2011](#); [U.S. EPA, 2006b](#)), an empirical basis for interpreting hair Pb measurements in terms of body burden or exposure has not been firmly established.

3.3.4.3 Saliva

A growing body of literature on the utility of measurements of salivary Pb has developed since the completion of the 2006 Pb AQCD ([U.S. EPA, 2006b](#)). Earlier reports suggested a relatively strong correlation between salivary Pb concentration and blood Pb concentration ([Omokhodion and Crockford, 1991](#); [Brodeur et al., 1983](#); [P'an, 1981](#)); however, more recent assessments have shown relatively weak or inconsistent associations ([Costa de Almeida et al., 2011](#); [Costa de Almeida et al., 2010](#); [Costa de Almeida et al., 2009](#); [Barbosa et al., 2006b](#); [Nriagu et al., 2006](#)). The differences in these outcomes may reflect differences in blood Pb concentrations, exposure history and/or dental health (i.e., transfer of Pb between dentin and saliva) and possibly methods for determining Pb in saliva. Barbosa et al. ([2006b](#)) found a significant but relatively weak correlation (log[blood Pb] versus log[saliva Pb], $r = 0.277$, $p = 0.008$) in a sample of adults, ages 18-60 years ($N = 88$). The correlation was similar for salivary and plasma Pb. Nriagu et al. ([2006](#)) found also found a relatively weak association ($R^2 = 0.026$) between blood Pb (µg/dL) and salivary Pb (µg/L) in a sample of adults who resided in Detroit, MI ($N = 904$). Costa de Almeida et al. ([2009](#)) found a significant correlation between

salivary and blood Pb concentrations in children in a Pb-contaminated region in Sao Paulo State, Brazil ($r = 0.76$, $p = 0.04$, $N = 7$) prior to site remediation; however, the correlation degenerated ($r = 0.03$, $p = 0.94$, $N = 9$) following remediation. Nevertheless, salivary Pb concentrations in the group of children who lived in the contaminated area were significantly elevated compared to a reference population. It is possible, that salivary Pb measurements may be a useful non-invasive biomarker for detecting elevated Pb exposure; however, it is not clear based on currently available data, if salivary Pb measurements would be a more reliable measure of exposure than blood Pb measurements.

3.3.4.4 Serum δ -ALA and ALAD

The association between blood Pb and blood ALAD activity and serum δ -aminolevulinic acid (δ -ALA) levels was recognized decades ago as having potential use as a biomarker of Pb exposure ([Mitchell et al., 1977](#); [Hernberg et al., 1970](#)). More recently reference values for blood ALAD activity ratio (the ratio of ALAD activity in the blood sample to that measured after fully activating the enzyme in the sample) have been reported ([Gultepe et al., 2009](#)). Inhibition of erythrocyte ALAD by Pb results in a rise in the plasma concentration of the ALAD substrate δ -ALA. The δ -ALA biomarker can be measured in serum and has been used as a surrogate for Pb measurements in studies in which whole blood samples or adequately prepared plasma or serum samples were not available for Pb measurements ([Opler et al., 2008](#); [Opler et al., 2004](#)).

3.3.5 Relationship between Pb in Blood and Pb in Bone

The kinetics of elimination of Pb from the body reflects the existence of multiple pools of Pb in the body that have different elimination kinetics. The dominant washout phase of Pb from the blood, exhibited shortly after a change in exposure occurs, has a half-life of ~20-30 days ([Leggett, 1993](#); [Rabinowitz et al., 1976](#)). Studies of a limited number of adults (four individuals with hip or knee replacement, a married couple, and 10 female Australian immigrants) in which the Pb exposure was from historical environmental sources (i.e., minimal current Pb exposure relative to past Pb exposure) have found that bone Pb stores can contribute as much as 40-70% to blood Pb ([Smith et al., 1996](#); [Gulson et al., 1995a](#); [Manton, 1985](#)). Bone Pb burdens in adults are slowly lost by diffusion (heteroionic exchange) as well as by bone resorption ([O'Flaherty, 1995](#)). Half-times for the release of Pb in bone are dependent on age and intensity of exposure. Bone compartments are much more labile in infants and children than in adults as reflected by

half-times for movement of Pb from bone into the plasma (e.g., cortical $t_{1/2}$ = 0.23 years at birth, 1.2 years at 5 years of age, 3.7 years at 15 years of age, and 23 years in adults; trabecular $t_{1/2}$ = 0.23 years at birth, 1.0 years at 5 years of age, 2.0 years at 15 years of age, and 3.9 years in adults) ([Leggett, 1993](#)). Slow transfer rates for the movement of Pb from nonexchangeable bone pools to the plasma are the dominant transfer process determining long-term accumulation and elimination of bone Pb burden.

When blood Pb concentrations are monitored in individuals over periods of years following a cessation or decrease in exposure, the decrease in blood Pb concentration exhibits complex kinetics that can be disaggregated into components having faster and slower rates. The slower rates of clearance of Pb from the blood over months and years following the cessation or reduction in exposures is thought to primarily reflect elimination of Pb stores in bone. Nilsson et al. ([1991](#)) reported a tri-exponential decay in the blood Pb concentrations of 14 individuals having a median occupational exposure period of 26 years. Thirteen of these 14 individuals had been temporarily removed from work because of excessive exposures (blood levels ≥ 70 $\mu\text{g/dL}$ or high urinary δ -aminolevulinic acid levels). Representing 22% of blood Pb, the fast compartment had a clearance half time of 34 days. The intermediate compartment, 27% of blood Pb, had a clearance half time of 1.12 year. And, the slow compartment, 50% of blood Pb, had a clearance half time of 13 years. The authors attributed the fast, intermediate, and slow compartment clearance to elimination of Pb from blood and some soft tissues, from trabecular bone, and cortical bone, respectively. Rentschler et al. ([2012](#)) also observed a slow terminal phase of Pb elimination from blood in five adults who had Pb poisoning due to either occupational or non-occupational exposures that ranged from approximately 1 month to 12 years and resulted in blood Pb concentrations of 70-110 $\mu\text{g/dL}$. In this study, the blood Pb monitoring period extended from 1 to 74 days following cessation of exposure to approximately 800 days following the diagnosis of poisoning; however, it was not of sufficient duration to estimate the terminal half-time. When the terminal half-time estimated by Nilsson et al. ([1991](#)) was used (13 years) to fit data for these Pb poisoning cases to a two-component exponential decay model, the initial faster phase represented approximately 80% of the blood Pb and the half-time was estimated to range from 60 to 120 days. The relatively longer fast phase half-time reported by Rentschler et al. ([2012](#)) compared to Nilsson et al. ([1991](#)) may reflect the relatively high blood Pb concentrations in these poisoning cases that resulted in temporary anemia and subsequent reestablishment of a normal erythrocyte levels. Also, the use of a two-compartment model, with an assumed slow half-time of 13 years, as well as uncertainty about the actual time of cessation of exposure may have prevented discerning a third, faster elimination compartment in these data.

Children who have been removed from a relatively brief exposure to elevated environmental Pb also exhibit faster slow-phase kinetics than children removed from exposures that lasted several years, with half-times of 10 and 20-38 months, respectively ([Manton et al., 2000](#)). Rothenberg et al. ([1998](#)) also showed that exposures in the first 6 months of life could contribute to elevated blood Pb levels through at least 3 years relative to children with lower early life exposures, despite similar environmental exposures at later time points. In both adults and children, the longer half-times measured under the latter conditions reflect the contribution of bone Pb stores to blood Pb following a change in exposure.

The longer half-life of Pb in bone compared to blood Pb, allows a more cumulative measure of long-term Pb exposure. Pb in adult bone can serve to maintain blood Pb levels long after external exposure has ceased ([Fleming et al., 1997](#); [Inskip et al., 1996](#); [Smith et al., 1996](#); [Kehoe, 1987](#); [O'Flaherty et al., 1982](#)), even for exposures that occurred during childhood ([McNeill et al., 2000](#)). The more widespread use of in vivo XRF Pb measurements in bone and indirect measurements of bone processes with stable Pb isotopes have enhanced the use of bone Pb as a biomarker of Pb body burden.

Several studies have found a stronger relationship between patella Pb and blood Pb than tibia Pb and blood Pb ([Park et al., 2009b](#); [Hu et al., 1998](#); [Hernandez-Avila et al., 1996](#); [Hu et al., 1996a](#)). Hu et al. ([1998](#)) suggest that trabecular bone is the predominant bone type providing Pb back into circulation under steady-state and pathologic conditions. The stronger relationship between blood Pb and trabecular Pb compared with cortical bone is probably associated with the larger surface area of trabecular bone allowing for more Pb to bind via ion exchange mechanisms and more rapid turnover making it more sensitive to changing patterns of exposure.

Relationships between Pb in blood and bone in children and adults are discussed in greater detail below ([Section 3.3.5.1](#), and [Section 3.3.5.2](#)). In these discussions, simulations based on a biokinetics model are shown to illustrate general patterns in the relationships between bone Pb and blood Pb that can be predicted based on the current understanding of Pb biokinetics in children and adults. However, these simulations reflect assumptions in the model and may not accurately represent the observed blood Pb kinetics in individuals or variability in blood Pb kinetics observed in specific populations. The simulations include two metrics of blood Pb, the blood Pb concentration at each time point in the simulation and the time-integrated blood Pb for the period preceding each time point in the simulation (also referred to as the cumulative blood Pb index [CBLI]). The time-integrated blood Pb metric has been used to estimate long-term average and cumulative absorbed Pb doses in epidemiologic studies (e.g., [Nie et al., 2011b](#); [Healey et](#)

[al., 2008](#); [Hu et al., 2007a](#); [Chuang et al., 2000](#); [McNeill et al., 2000](#); [Fleming et al., 1997](#); [Roels et al., 1995](#); [Gerhardsson et al., 1993](#); [Armstrong et al., 1992](#)).

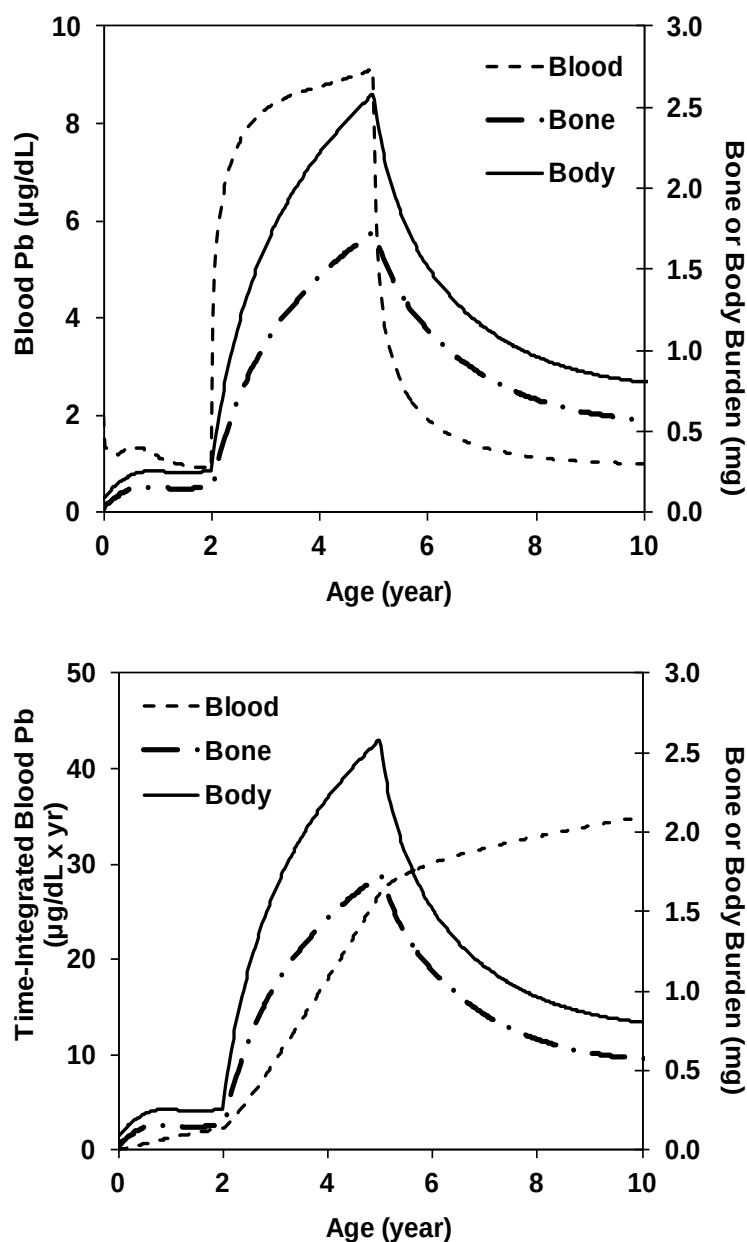
3.3.5.1 Children

As mentioned in [Section 3.2.2.2](#), bone growth in children will contribute to accumulation of Pb in bone, which will comprise most of the Pb body burden. As a result, Pb in bone will more closely reflect Pb body burden than blood Pb. However, changes in blood Pb concentration in children (i.e., associated with changing exposures) are thought to more closely parallel changes in total body burden. [Figure 3-9](#) shows a biokinetics model simulation of the temporal profile of Pb in blood and bone in a child who experiences a period of constant Pb intake (from age 2-5) via ingestion ($\mu\text{g Pb/day}$) followed by an abrupt decline in intake. The figure illustrates several important general concepts about the relationship between Pb in blood and bone. While blood Pb approaches a quasi-steady state after a period of a few months with a constant rate of Pb intake (as demonstrated by the vertical dashed line), Pb continues to accumulate in bone with continued Pb intake after the quasi-steady state is achieved in blood. The model also predicts that the rate of release of Pb from bone after a reduction in exposure is faster than in adults. This difference has been attributed to accelerated growth-related bone mineral turnover in children, which is the primary mechanism for release of Pb that has been incorporated into the bone mineral matrix.

Empirical evidence in support of this conclusion comes from longitudinal studies in which relatively high correlations were found between concurrent ($r = 0.75$) or average lifetime (obtained at 6-month intervals from birth to age 10 or 12) blood Pb concentrations ($r = 0.85$) and tibia bone Pb concentrations (measured by XRF) in a sample of children in which the group mean concurrent blood Pb concentration exceeded $20 \mu\text{g/dL}$; the correlations was much weaker ($r < 0.15$) among the group of children with a mean concurrent blood Pb concentration $< 10 \mu\text{g/dL}$ ([Wasserman et al., 2003](#)).

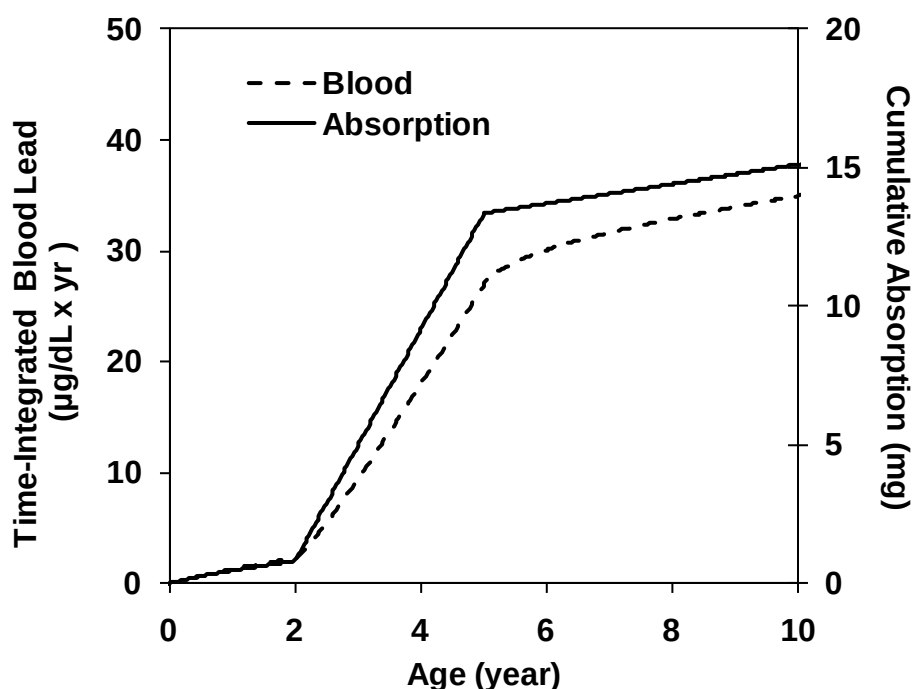
Time-integrated blood Pb metrics display rates of change in response to the exposure event that more closely approximate the slower kinetics of total skeletal bone Pb and body burden, than the kinetics of blood Pb concentration, with notable differences ([Figure 3-9](#)). The time-integrated blood Pb concentration is a cumulative function and increases throughout childhood; however, the slope of the increase is higher during the exposure event than prior to or following the event. Following cessation of the enhanced exposure period, the time-integrated blood Pb and body burden diverge. This result is expected, as the time-integrated blood Pb curve is a cumulative function which cannot decrease over time and bone Pb levels will decrease with reduction in exposure.

The time-integrated blood Pb concentration will be a better reflection of the total amount of Pb that has been absorbed, than the body burden at any given time. The time-integrated blood Pb concentration will also reflect cumulative Pb absorption, and cumulative exposure if the absorption fraction is constant. This concept is illustrated in the hypothetical simulations of an exposure event experienced by a child ([Figure 3-10](#)). This pattern is similar for adults.



Note: Blood Pb concentration is thought to parallel body burden more closely in children than in adults, due to more rapid turnover of bone and bone-Pb stores in children (upper panel). Baseline Pb intake is 3.2 µg/day from birth until age 2, followed by a period of increased intake (38.2 µg/day) from age 2 until age 5, with a return to baseline intake of 3.2 µg/day at age 5. The time-integrated blood Pb concentration increases over time (lower panel). Simulation based on ICRP Pb biokinetics model ([Leggett, 1993](#)).

Figure 3-9 **Simulation of relationship between blood Pb concentration and body burden in children, with an elevated constant Pb intake from age 2 to 5 years.**



Note: The simulations include a 3-year period of elevated constant Pb intake during ages 2-5 years. Baseline Pb intake is 3.2 µg/day from birth until age 2, followed by a period of increased intake (38.2 µg/day) from age 2 until age 5, with a return to baseline intake of 3.2 µg/day at age 5. The time-integrated blood Pb concentration closely parallels cumulative Pb absorption. Simulation based on ICRP Pb biokinetics model ([Leggett, 1993](#)).

Figure 3-10 **Simulation of relationship between time-integrated blood Pb concentration and cumulative Pb absorption in children.**

3.3.5.2 **Adults**

In adults, where a relatively large fraction of the body burden residing in bone has a slower turnover compared to blood, a constant Pb uptake (or constant intake and fractional absorption) gives rise to a quasi-steady state blood Pb concentration, while the body burden continues to increase over a much longer period, largely as a consequence of continued accumulation of Pb in bone. This pattern is illustrated in [Figure 3-11](#) that depicts a hypothetical simulation of an exposure consisting of a 20-year period of daily ingestion of Pb in an adult. The exposure shown in the simulations gives rise to a relatively rapid increase in blood Pb concentration from a baseline of approximately 2 µg/dL, to a new quasi-steady state of approximately 9 µg/dL, achieved in ~75-100 days (i.e., approximately 3-4 times the blood elimination half-life). In contrast, the body burden exhibits a steady increase across the full exposure period of 70 years. Following cessation of the enhanced exposure period, blood Pb concentration declines relatively

rapidly compared to the slower decline in body burden. Careful examination of the simulation shown in [Figure 3-11](#) reveals that the accumulation and elimination phases of blood Pb kinetics are not symmetrical; elimination is slower than accumulation as a result of the gradual release of bone Pb stores to blood. This response, known as the prolonged terminal elimination phase of Pb from blood, has been observed in retired Pb workers and in workers who continued to work after improved industrial hygiene standards reduced their exposures. Based on this hypothetical simulation, a blood Pb concentration measured 1 year following cessation of a period of increased Pb uptake would be elevated by only a relatively small amount over the baseline measured prior to the exposure (3 µg/dL versus the 2 µg/dL), whereas, the body burden would remain elevated. These simulations in [Figure 3-11](#) illustrate how a single blood Pb concentration measurement, or a series of measurements taken over a short-time span, could be a relatively poor index of Pb body burden.

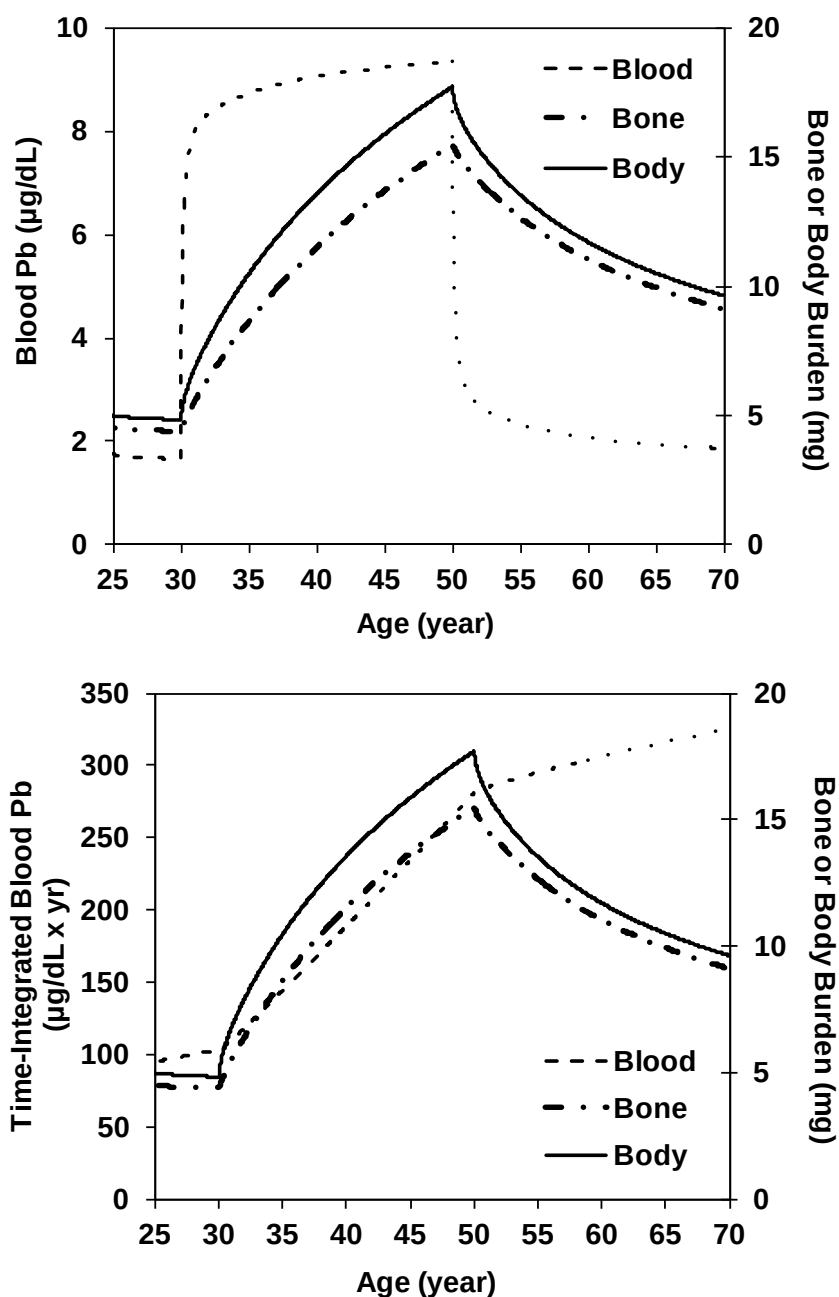
The simulation shown in [Figure 3-11](#) represents an exposure that resulted in a quasi-steady state blood Pb concentration of approximately 10 µg/dL. Exposures that achieve higher blood Pb concentrations, more indicative of poisoning or historic occupational exposures will result in a more prolonged elevation of blood Pb concentration following cessation of the enhanced exposure period. [Figure 3-12](#) shows a model simulation of an adult exposed to Pb, that results in a quasi-steady state blood Pb concentration of approximately 90 µg/dL. In this case, the blood Pb concentration remains substantially elevated 1 year following the exposure event (42 µg/dL versus 2 µg/dL) and 20 years following the exposure event (11 µg/dL).

The drop in blood Pb concentrations following cessation of elevated exposure in [Figure 3-11](#) and [Figure 3-12](#) are well described ($r = 0.996$) by a tri-exponential decay function having the half-times of 30 days, 5 months, and 8 years for the fast, intermediate, and slow compartments, respectively. For the low level of Pb intake illustrated in [Figure 3-11](#), the fast, intermediate, and slow clearance compartments represent 66%, 19%, and 15% of blood Pb, respectively. For the high level of Pb intake illustrated in [Figure 3-12](#), the fast, intermediate, and slow clearance compartments represent 35%, 19%, and 46% of blood Pb, respectively. The higher exposure resulted in more accumulation of Pb in bone relative to the lower exposure scenario. This bone accumulation is reflected in the blood Pb clearance kinetics by a larger slow compartment and smaller fast compartment for the high exposure in [Figure 3-12](#) relative to the lower exposure in [Figure 3-11](#).

One important potential implication of the profoundly different kinetics of Pb in blood and bone is that, for a constant Pb exposure, Pb in bone will increase with increasing duration of exposure and, therefore, with age. In contrast, blood Pb concentration will achieve a quasi-steady state. As a result, the relationship between blood Pb and bone Pb

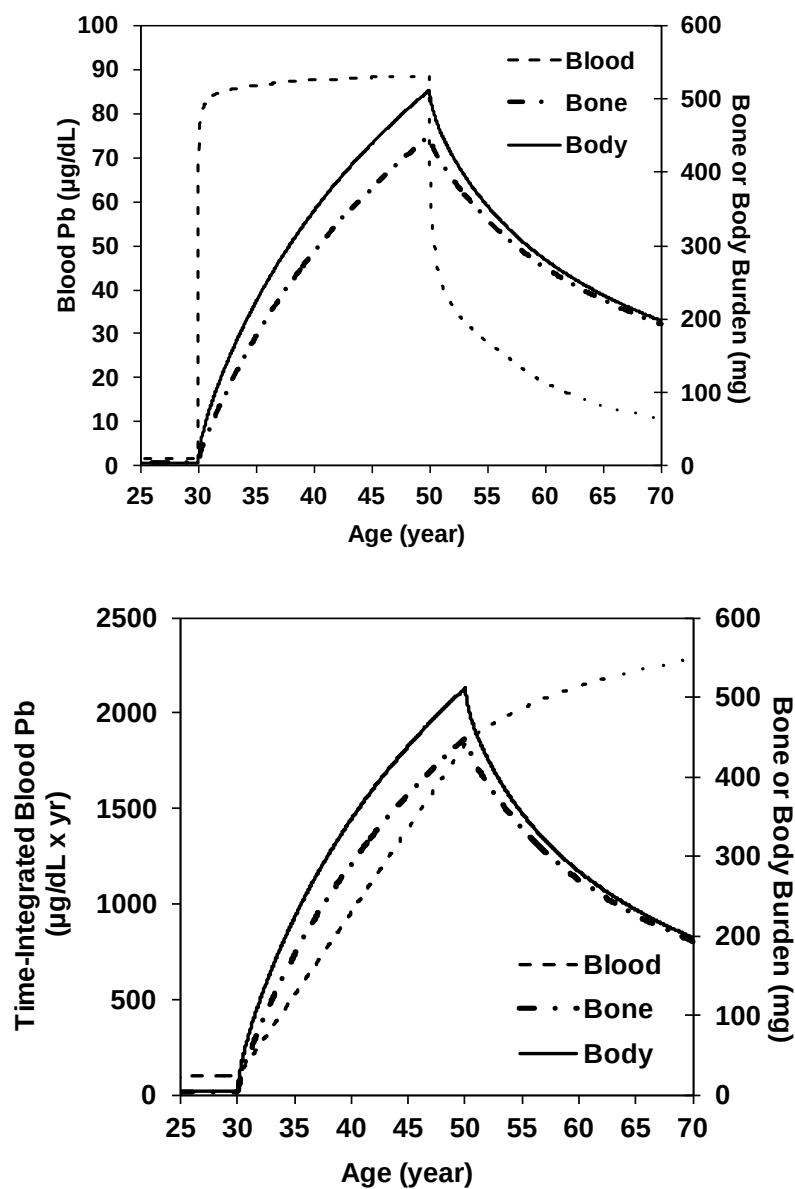
will diverge with increasing exposure duration and age. This divergence can impart different degrees of age-confounding when either blood Pb or bone Pb is used as an internal dose metric in dose-response models. In a review of epidemiologic studies that evaluated the associations between blood Pb, bone Pb and cognitive function, the association was stronger for bone Pb than blood Pb (particularly for longitudinal studies) for older individuals with environmental Pb exposures and low blood Pb levels ([Shih et al., 2007](#)). In contrast, occupational workers with high current Pb exposures had the strongest associations for blood Pb levels with cognitive function, thus providing evidence for this divergence ([Shih et al., 2007](#)).

The aforementioned expectation for an increase in bone Pb and body burden with age applies to scenarios of constant exposure but not necessarily to real world populations in which individual and population exposures have changed over time. Longitudinal studies of blood and bone Pb trends have not always found strong dependence on age ([Nie et al., 2009](#); [Kim et al., 1997](#)). Kim et al. ([1997](#)) found that bone Pb levels increased with increasing age in elderly adults (age 52-83) years), only when the data were analyzed cross-sectionally. When analyzed longitudinally, the trend for individual patella Pb was a 23% decrease over a 3-year period (approximate $t_{1/2}$ of 8 years), whereas tibia Pb levels did not change with over the same period. Therefore, although older individuals tended to have higher bone Pb levels, the 3-year temporal trend for individuals was a loss of Pb from the more labile Pb stores in trabecular bone. Nie et al. ([2011a](#)) observed that longitudinal observations of blood and bone Pb in elderly adults did not show a significant age effect on the association between blood Pb and bone Pb (patella and tibia), when the sample population (N=776) was stratified into age tertiles (mean age 62, 69 or 77 years). Nie et al. ([2009](#)) did find that regressed function bone Pb and appeared to level off at bone Pb levels >20 µg/g bone mineral.



Note: A constant baseline intake of 20 µg/day from age 0-30 results in a quasi-steady state blood Pb concentration and body burden. An increase in Pb intake to 120 µg/day from age 30 to 50 gives rise to a relatively rapid increase in blood Pb, to a new quasi-steady state, and a slower increase in body burden (upper panel). At age 50, intake returns to the baseline of 20 µg/day. Following the long period of elevated Pb intake, there is a rapid decline in blood Pb from 9 to 3 µg/dL over the first year and a more gradual decline in blood Pb to less than 2 µg/dL by age 60. The time-integrated blood Pb concentration increases over the lifetime, with a greater rate of increase during periods of higher Pb uptake (lower panel). Simulation based on ICRP Pb biokinetics model ([Leggett, 1993](#)).

Figure 3-11 Simulation of relationship between blood Pb concentration, bone Pb and body burden in adults with relatively low Pb intake.



Note: A constant baseline intake of 20 µg/day from age 0-30 results in a quasi-steady state blood Pb concentration and body burden. An increase in Pb intake to 6020 µg/day from age 30 to 50 gives rise to a relatively rapid increase in blood Pb, to a new quasi-steady state, and a slower increase in body burden (upper panel). At age 50, intake returns to the baseline of 20 µg/day. Following the long period of high Pb intake, there is a rapid decline in blood Pb from 90 to 40 µg/dL over the first a year followed by a more gradual decline in blood Pb to 20 µg/dL by age 60 and 10 µg/dL at age 70. The time-integrated blood Pb concentration increases over the lifetime, with a greater rate of increase during periods of higher Pb uptake (lower panel). Simulation based on ICRP Pb biokinetics model ([Leggett, 1993](#)).

Figure 3-12 Simulation of relationship between blood Pb concentration, bone Pb and body burden in adults with relatively high Pb intake.

Tibia bone Pb is correlated with time-integrated blood Pb concentration (i.e., CBLI). McNeill et al. ([2000](#)) compared tibia Pb levels and cumulative blood Pb indices in a population of 19- to 29-year-olds who had been highly exposed to Pb in childhood from the Bunker Hill, Idaho smelter. They concluded that Pb from exposure in early childhood had persisted in the bone matrix until adulthood. The bone Pb/CBLI slopes from various studies range from 0.022 to 0.067 $\mu\text{g/g}$ bone mineral per $\mu\text{g-year/dL}$ ([Healey et al., 2008](#); [Hu et al., 2007a](#)). Because the CBLI is a cumulative function which cannot decrease over time, CBLI and bone Pb would be expected to diverge following cessation of exposure, as bone Pb levels decrease. This divergence was observed as a lower bone Pb/CBLI slope in retired Pb workers compared to active workers and in worker populations whose exposures declined over time as a result of improved industrial hygiene ([Fleming et al., 1997](#); [Gerhardsson et al., 1993](#)).

Although differences in kinetics of blood and bone Pb degrade the predictive value of blood Pb as a metric of Pb body burden, within a population that has similar exposure histories and age demographics, blood and bone Pb may show relatively strong associations. A recent analysis of a subset of data from the Normative Aging Study (an all male cohort) showed that cross-sectional measurements of blood Pb concentration accounted for approximately 9% (tibia) to 13% (patella) of the variability in bone Pb levels. Inclusion of age in the regression model accounted for an additional 7-10% of the variability in bone Pb ([Park et al., 2009b](#)).

Mobilization of Pb from Bone in Adulthood

In addition to changes in exposure (e.g., declines in exposure discussed in prior sections), there are physiological processes during different life circumstances that can increase the contribution of bone Pb to blood Pb. These life circumstances include times of physiological stress associated with enhanced bone remodeling such as during pregnancy and lactation ([Hertz-Picciotto et al., 2000](#); [Silbergeld, 1991](#); [Manton, 1985](#)), menopause or in the elderly ([Silbergeld et al., 1988](#)), extended bed rest ([Markowitz and Weinberger, 1990](#)), hyperparathyroidism ([Kessler et al., 1999](#)) and severe weight loss ([Riedt et al., 2009](#)).

During pregnancy, bone Pb can serve as a Pb source as maternal bone is resorbed for the production of the fetal skeleton ([Gulson et al., 2003](#); [Gulson et al., 1999](#); [Franklin et al., 1997](#); [Gulson et al., 1997](#)). Increased blood Pb during pregnancy has been demonstrated in numerous studies and these changes have been characterized as a “U-shaped” pattern of lower blood Pb concentrations during the second trimester compared to the first and third trimesters ([Lamadrid-Figueroa et al., 2006](#); [Gulson et al., 2004a](#); [Hertz-Picciotto et al., 2000](#); [Gulson et al., 1997](#); [Lagerkvist et al., 1996](#); [Schuhmacher et al., 1996](#);

[Rothenberg et al., 1994a](#)). The U-shaped relationship reflects the relatively higher impact of hemodilution in the second trimester versus the rate of bone Pb resorption accompanying Ca^{2+} releases for establishing the fetal skeleton. In the third trimester, fetal skeletal growth on calcium demand is greater, and Pb released from maternal skeleton offsets hemodilution. Gulson et al. ([1998b](#)) reported that, during pregnancy, blood Pb concentrations in the first immigrant Australian cohort (N = 15) increased by an average of about 20% compared to non-pregnant migrant controls (N = 7). Skeletal contribution to blood Pb, based on the isotopic composition for the immigrant subjects, increased in an approximately linear manner during pregnancy. The mean increases for each individual during pregnancy varied from 26% to 99%. Interestingly, the percent change in blood Pb concentration was significantly greater during the post-pregnancy period than during the second and third trimesters. This is consistent with Hansen et al. ([2011b](#)) that demonstrated the greatest blood Pb levels at 6 weeks postpartum compared to the second trimester in 211 Norwegian women. Increased calcium demands of lactation (relative to pregnancy) may contribute to the greater change in blood Pb observed post pregnancy compared to the second and third trimesters. The contribution of skeletal Pb to blood Pb during the post-pregnancy period remained essentially constant at the increased level of Pb mobilization.

Gulson et al. ([2004a](#)) observed that calcium supplementation was found to delay increased mobilization of Pb from bone during pregnancy and halved the flux of Pb release from bone during late pregnancy and postpartum. In another study, women whose daily Ca^{2+} intake was 850 mg per day showed lower amounts of bone resorption during late pregnancy and postpartum than those whose intake was 560 mg calcium per day ([Manton et al., 2003](#)). Similarly, calcium supplementation (1,200 mg/day) in pregnant Mexican women resulted in an 11% reduction in blood Pb level compared to placebo and a 24% average reduction for the most compliant women ([Ettinger et al., 2009](#)). When considering baseline blood Pb levels in women who were more compliant in taking calcium supplementation, the reductions were similar for those $<5 \mu\text{g/dL}$ and those $\geq 5 \mu\text{g/dL}$ (14% and 17%, respectively). This result is in contrast to a study of women who had blood Pb concentrations $<5 \mu\text{g/dL}$, where calcium supplementation had no effect on blood Pb concentrations ([Gulson et al., 2006b](#)). These investigators attributed their results to changes in bone resorption with decoupling of trabecular and cortical bone sites.

Miranda et al. ([2010](#)) studied blood Pb level among pregnant women aged 18-44 years old. The older age segments in the study presumably had greater historic Pb exposures and associated stored Pb than the younger age segments. Compared with the blood Pb levels of a reference group in the 25-29 years old age category, pregnant women ≥ 30 years old had significant odds of having higher blood Pb levels (aged 30-34:

OR = 2.39, $p < 0.001$; aged 35-39: OR = 2.98, $p < 0.001$; aged 40-44: OR = 7.69, $p < 0.001$). Similarly, younger women had less chance of having higher blood Pb levels compared with the reference group (aged 18-19: OR = 0.60, $p = 0.179$; aged 20-24: OR = 0.54, $p = 0.015$). These findings indicate that maternal blood Pb levels are more likely the result of Pb mobilization of bone stores from historic exposures as opposed to contemporaneous exposures.

Blood Pb levels increase during lactation due to alterations in the endogenous bone Pb release rate. After adjusting for patella Pb concentration, an increase in blood Pb levels of 12.7% (95% CI: 6.2, 19.6) was observed for women who practiced partial lactation and an increase of 18.6% (95% CI: 7.1, 31.4) for women who practiced exclusive lactation compared to those who stopped lactation ([Tellez-Rojo et al., 2002](#)). In another Mexico City study, Ettinger et al. ([2006](#); [2004b](#)) concluded that an interquartile increase in patella Pb was associated with a 14% increase in breast milk Pb, whereas for tibia Pb the increase was ~5%. Breast milk:maternal blood Pb concentration ratios are generally < 0.1 , although values of 0.9 have been reported ([Koyashiki et al., 2010](#); [Ettinger et al., 2006](#); [Gulson et al., 1998a](#)). Dietary intake of polyunsaturated fatty acids (PUFA) has been shown to weaken the association between Pb levels in patella and breast milk, perhaps indicating decreased transfer of Pb from bone to breast milk with PUFA consumption ([Arora et al., 2008](#)). Breast milk as a source of infant Pb exposure was also discussed in [Section 3.1.3.3](#) on dietary Pb exposure.

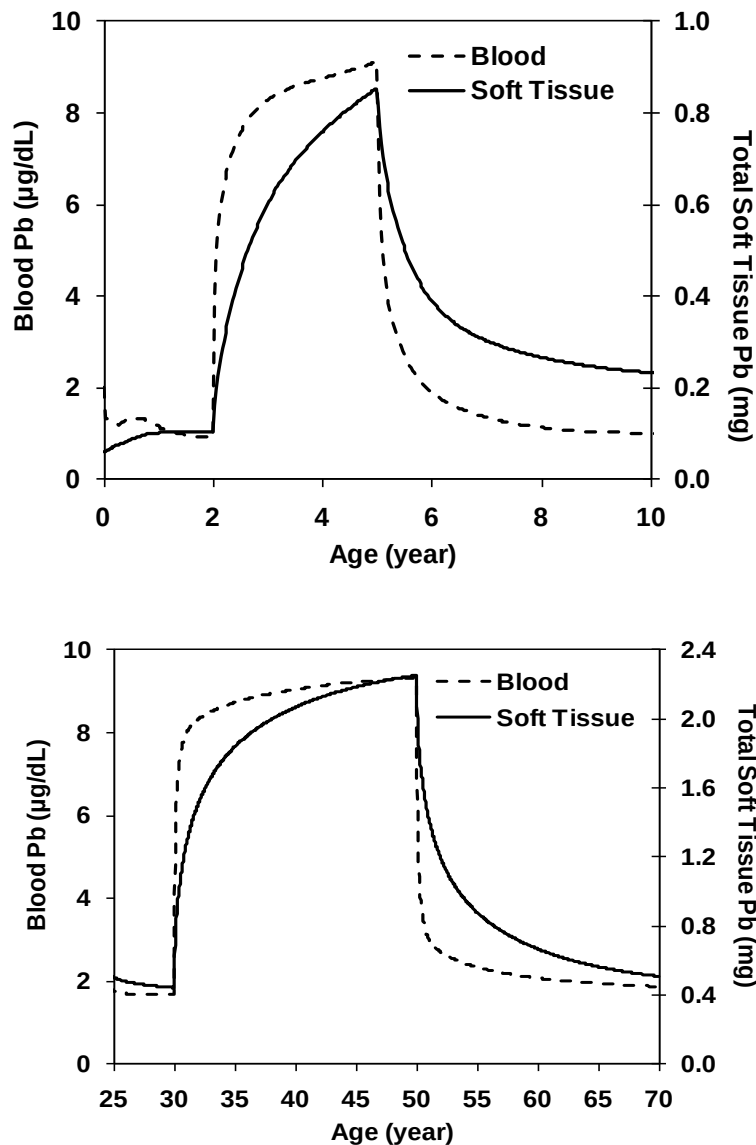
The Pb content in some bones (i.e., mid femur and pelvic bone) plateau at middle age and then decreases at older ages ([Drasch et al., 1987](#)). This decrease is most pronounced in females and may be due to osteoporosis and release of Pb from resorbed bone to blood ([Gulson et al., 2002](#)). Two studies indicate that the endogenous release rate in postmenopausal women ranges from 0.13-0.14 $\mu\text{g/dL}$ in blood per $\mu\text{g/g}$ bone and is nearly double the rate found in premenopausal women (0.07-0.08 $\mu\text{g/dL}$ per $\mu\text{g/g}$ bone) ([Popovic et al., 2005](#); [Garrido Latorre et al., 2003](#)). An analysis of data on blood Pb concentrations and markers of bone formation (serum alkaline phosphatase) and resorption (urinary cross-linked N-telopeptides, NTx) in a sample of U.S. found that blood Pb concentrations were higher in women (pre- or post-menopausal) who exhibited the highest bone formation or resorption activities ([Jackson et al., 2010](#)). Calcium or vitamin D supplementation decreased the blood Pb concentrations in the highest bone formation and resorption tertiles of the population of post-menopausal women. Significant associations between increasing NTx and increasing blood Pb levels (i.e., increased intercept of regression model relating the change in blood Pb per change in bone Pb) have also been observed in elderly males ([Nie et al., 2009](#)).

Studies of the effect of hormone replacement therapy on bone Pb mobilization have yielded conflicting results ([Popovic et al., 2005](#); [Berkowitz et al., 2004](#); [Garrido Latorre et al., 2003](#); [Korrick et al., 2002](#); [Webber et al., 1995](#)). In women with severe weight loss (28% of BMI in 6 months) sufficient to increase bone turnover, increased blood Pb levels of approximately 2.1 µg/dL (250%) were reported, and these blood Pb increases were associated with biomarkers of increased bone turnover (e.g., urinary pyridinoline cross-links) ([Riedt et al., 2009](#)).

3.3.6 Relationship between Pb in Blood and Pb in Soft Tissues

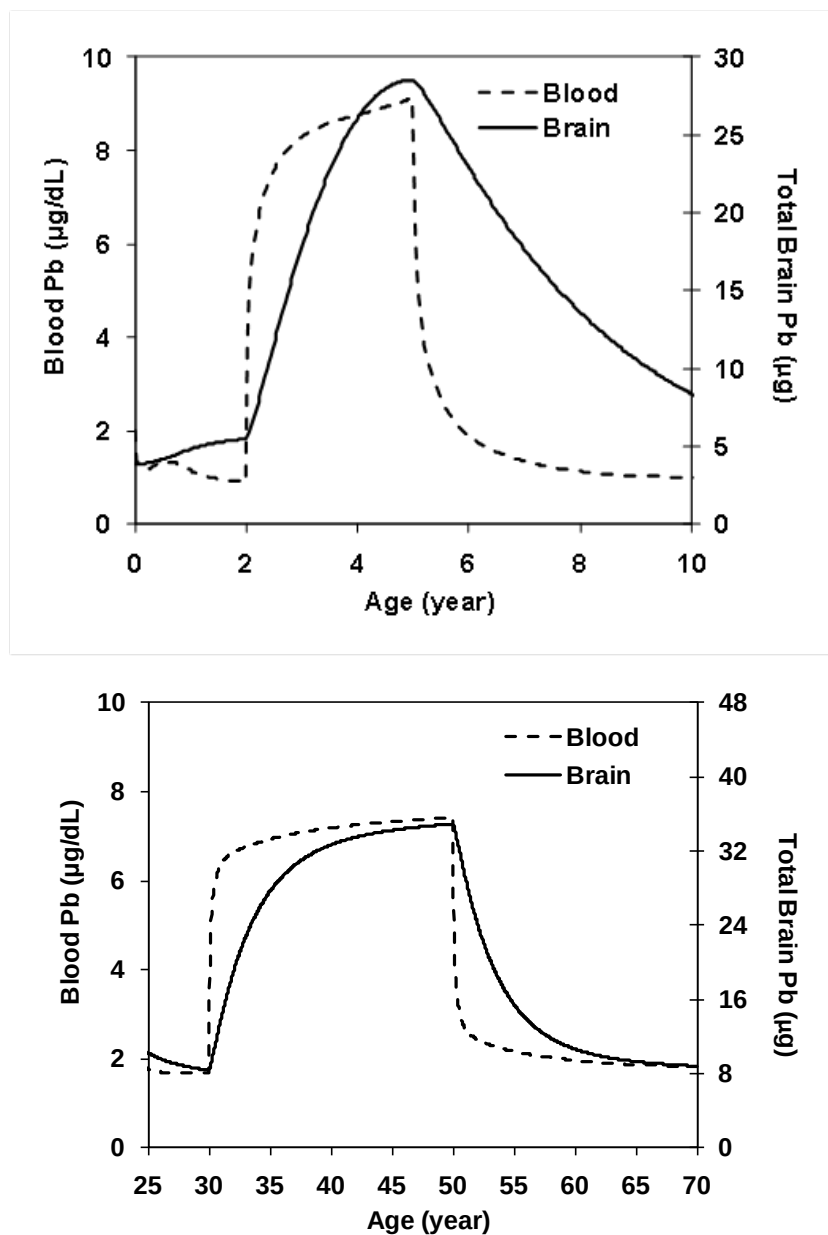
[Figure 3-13](#) shows simulations of blood and soft tissues Pb (including brain) for the same exposure scenarios previously displayed. Pb uptake and elimination in soft tissues is much faster than bone. As a result, following cessation of a period of elevated exposure, Pb in soft tissues is more quickly returned to blood. The terminal elimination phase from soft tissue mimics that of blood, and it is similarly influenced by the contribution of bone Pb returned to blood and being redistributed to soft tissue.

Information on Pb levels in human brain is limited to autopsy data. These data indicate brain/blood Pb ratios of approximately 0.5 in infancy which remain relatively constant over the lifetime (range 0.3 to 1.1) ([Barry, 1981, 1975](#)). The simulation of brain Pb shown in [Figure 3-14](#) reflects general concepts derived from observations made in non-human primates, dogs and rodents. These observations suggest that peak Pb levels in the brain are reached 6 months following a bolus exposure and within two months approximately 80% of steady state brain Pb levels are reached ([Leggett, 1993](#)). There is a relatively slow elimination of Pb from brain ($t_{1/2} \approx 2$ years) compared to other soft tissues ([Leggett, 1993](#)). This slow elimination rate is reflected in the slower elimination phase kinetics is shown in [Figure 3-14](#). Although in this model, brain Pb to blood Pb transfer half-times are assumed to be the same in children and adults, uptake kinetics are assumed to be faster during infancy and childhood, which achieves a higher fraction of the soft tissue burden in brain, consistent with higher brain/body mass relationships. The uptake half times predicted by Leggett ([1993](#)) vary from 0.9 to 3.7 days, depending on age. Brain Pb kinetics represented in the simulations are simple outcomes of modeling assumptions and cannot currently be verified with available observations in humans.



Note: For the child simulation (upper panel), baseline Pb intake is 3.2 µg/day from birth until age 2, followed by a period of increased intake to 38.2 µg/day from age 2 until 5, with a return to baseline intake at age 5. For the adult simulation (lower panel), baseline intake is 20 µg/day from age 0-30, followed by a 20-year period of increased intake to 120 µg/day from age 30 to 50, with a return to baseline intake at age 50. Simulation based on ICRP Pb biokinetics model ([Leggett, 1993](#)).

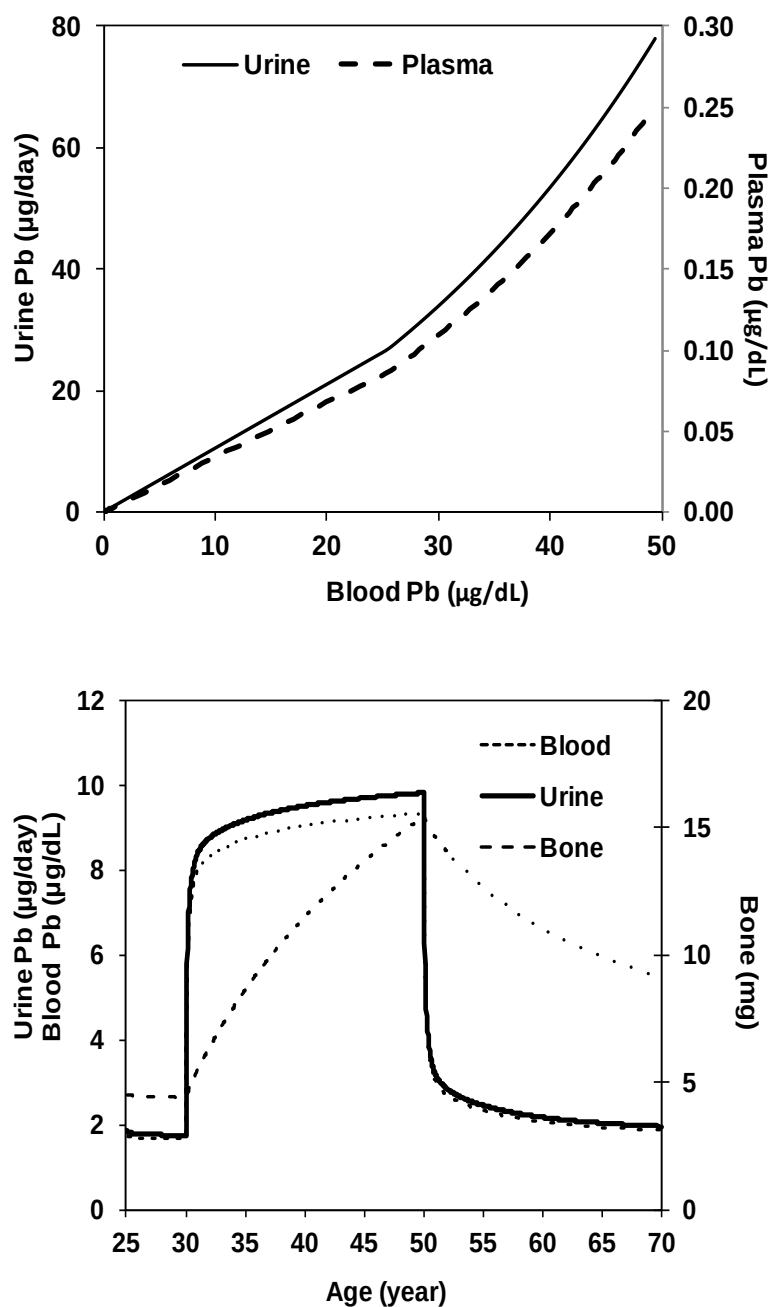
Figure 3-13 Simulation of blood and soft tissue (including brain) Pb in children and adults who experience a period of increased Pb intake.



Note: For the child simulation (upper panel), baseline Pb intake is 3.2 µg/day from birth until age 2, followed by a period of increased intake to 38.2 µg/day from age 2 until 5, with a return to baseline intake at age 5. For the adult simulation (lower panel), baseline intake is 20 µg/day from age 0-30, followed by a 20-year period of increased intake to 120 µg/day from age 30 to 50, with a return to baseline intake at age 50. Simulation based on ICRP Pb biokinetics model ([Leggett, 1993](#)).

Figure 3-14 Simulation of blood and brain Pb in children and adults who experience a period of increased Pb intake.

Urinary filtering and excretion of Pb is associated with plasma Pb concentrations. Given the curvilinear relationship between blood Pb and plasma Pb, a secondary expectation is for a curvilinear relationship between blood Pb and urinary Pb excretion that may become evident only at relatively high blood Pb concentrations (e.g., >25 µg/dL). [Figure 3-15](#) shows these relationships predicted from the model. In this case, the exposure scenario shown is for an adult (age 40 years) at a quasi-steady state blood Pb concentration; the same relationships hold for children ([Leggett, 1993](#)). At lower blood Pb concentrations (<25 µg/dL), urinary Pb excretion is predicted to closely parallel plasma Pb concentration for any given blood Pb level ([Figure 3-15](#), top panel). It follows from this that, similar to blood Pb, urinary Pb will respond much more rapidly to an abrupt change in Pb exposure than will bone Pb. One important implication of this relationship is that, as described previously for blood Pb, the relationships between urinary Pb and bone Pb will diverge with increasing exposure duration and age, even if exposure remains constant. Furthermore, following an abrupt cessation of exposure, urine Pb will quickly decrease while bone Pb will remain elevated ([Figure 3-15](#), lower panel).



Note: Upper panel, model simulations are for a 40-year old having a constant intake from birth of between 1 and 1,000 µg/day. For the lower panel, baseline intake is 20 µg/day from age 0-30, followed by a 20-year period of increased intake to 120 µg/day from age 30 to 50, with a return to baseline intake at age 50. Simulation based on ICRP Pb biokinetics model ([Leggett, 1993](#)).

Figure 3-15 Relationship between Pb in urine, plasma, blood and bone.

3.4 Studies of Pb Biomarker Levels

3.4.1 Pb in Blood

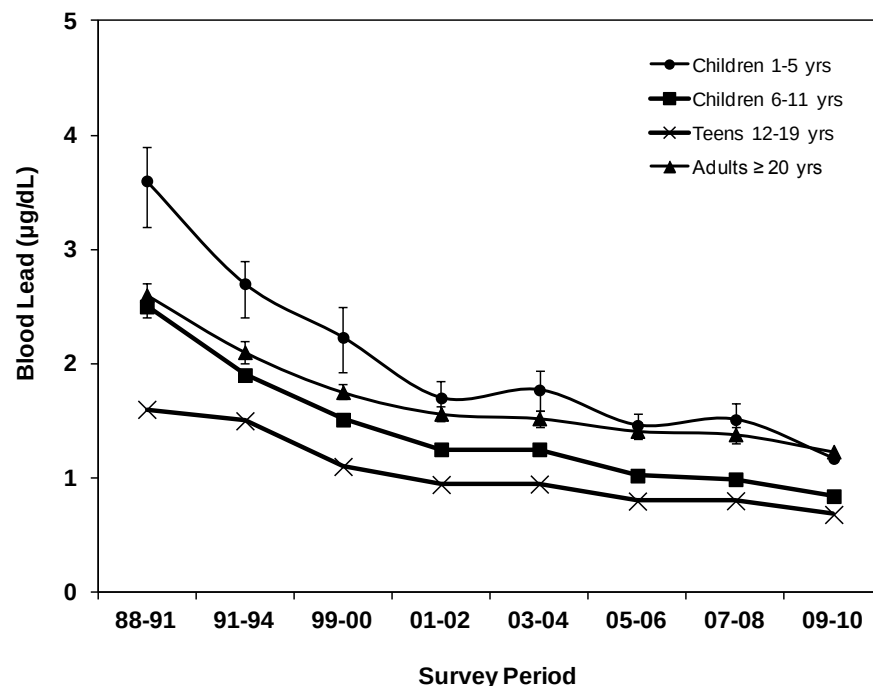
Overall, trends in blood Pb levels have been decreasing among U.S. residents over the past 35 years. Blood Pb concentrations in the U.S. general population have been monitored in the NHANES. Analyses of these data show a progressive downward trend in blood Pb concentrations during the period 1976-2010, with the most dramatic declines coincident with the phase out of leaded gasoline and reductions in point source Pb emissions described in [Section 2.2](#) ([Pirkle et al., 1998](#); [Brody et al., 1994](#); [Pirkle et al., 1994](#); [Schwartz and Pitcher, 1989](#)). The temporal trend for the period 1988-2010 is shown in [Figure 3-16](#). Summary statistics from the most recent publicly available data (1999-2010) are presented in [Table 3-8](#) ([CDC, 2013](#)). The geometric mean Pb concentration among children 1-5 years of age, based on the sample collected during the period 2009-2010, was 1.17 µg/dL (95% CI: 1.08, 1.26), which was decreased from 2007-2008 (1.51 µg/dL, 95% CI: 1.37, 1.66). [Figure 3-17](#) uses NHANES data to illustrate temporal trends in the distribution of blood Pb levels among U.S. children aged 1-5 years. For 2005-2010, the 95th percentile of blood Pb levels for children aged 1-5 years was less than 5 µg/dL. The geometric mean blood Pb concentration among adults ≥ 20 years of age was 1.23 µg/dL (95% CI: 1.19, 1.28) for the sample collected during the period 2009-2010 ([CDC, 2013](#)). Based on these same data, the geometric mean for all males (aged ≥ 1 year) was 1.31 µg/dL (95% CI: 1.25, 1.36), and for females (aged ≥ 1 year) was 0.97 µg/dL (95% CI: 0.93, 1.01).

There has been a steep decline in mean blood Pb levels from 1975 through 2010 among all birth cohorts from 1975 to 2010. [Figure 3-18](#) illustrates results of an analysis of data from the NHANES II (1976-1980), NHANES III (1988-1994), and continuous NHANES (1999-2008) surveys. In this analysis, participants were grouped by year of birth, ascertained by survey time period and reported age. The geometric mean of participant blood Pb was plotted for each age cohort as a function of year in which the survey was performed. For all cohorts, blood Pb generally decreases with age during childhood until adolescence; following adolescence (in the early 20s), blood Pb generally levels off or even increases with age. It is possible that bone growth in young people and occupational exposure for adults influences the shape of these curves. For the 1960 to 1970 birth cohort, the mean blood Pb is the highest of the cohorts in the 1970s, but beginning in 1993 the mean blood Pb is one of the lowest of the cohorts. This interaction between time and cohort may be due to the faster release of Pb from bone in younger people ([Rabinowitz, 1991](#)). This interaction is also apparent for some of the other more recently born cohorts. In comparison, the slopes of blood Pb over time are nearly parallel among

the cohorts born before 1960. This observation suggests that the time-cohort-interaction diminishes among older people. Also, the leveling of the blood Pb in the 2000s could be due to aging of the birth cohort and consequent slowing of their Pb release from bone.

When race/ethnicity groups were compared for years 1999-2004, geometric means (GM) of blood Pb levels in children were highest in the ethnicity category non-Hispanic black (GM 2.8, 95% CI: 2.5, 3.0) compared to the categories Mexican-American (GM 1.9, 95% CI: 1.7, 2.0) and non-Hispanic white (GM 1.7, 95% CI: 1.6, 1.8) ([Jones et al., 2009a](#)).

[Figure 3-19](#) demonstrates the change in percent of children (aged 1-5 years) with various blood Pb levels by race/ethnicity between the survey during 1988-1991 and that during 1999-2004. When these data for children aged 1-5 years were aggregated for all survey years from 1988 to 2004, residence in older housing, poverty, age, and being non-Hispanic black were significant predictors of higher Pb levels ([Jones et al., 2009a](#)).



Note: Shown are geometric means and 95% CIs based on data from NHANES III Phase 1 ([Brody et al., 1994](#); [Pirkle et al., 1994](#)); NHANES III Phase 2 ([Pirkle et al., 1998](#)); and NHANES IV ([CDC, 2011a](#)). Data for adults during the period 1988-1994 are for ages 20-49 years, and ≥ 20 years for the period 1999-2008.

Figure 3-16 Temporal trend in blood Pb concentration.

Table 3-8 Blood Pb concentrations in the U.S. population.

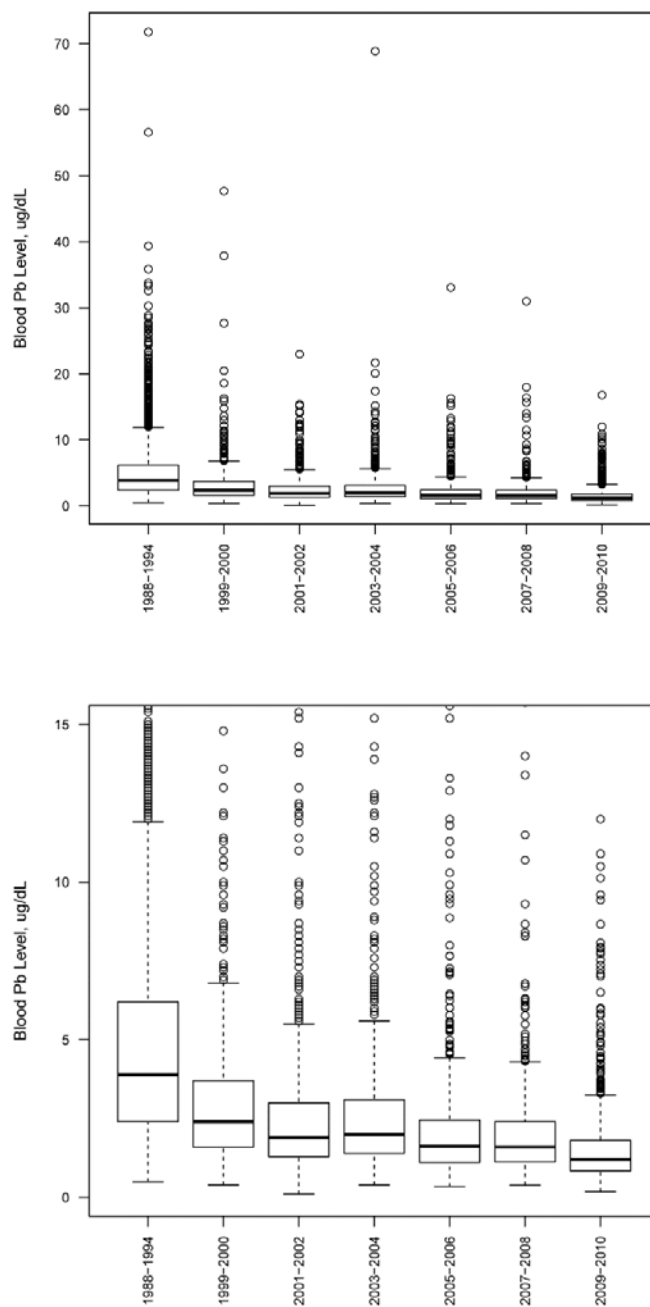
Survey Stratum	Period	Geometric Mean (µg/dL)	95% Confidence Interval	Number of Subjects
All	1999-2000	1.66	1.60, 1.72	7,970
	2001-2002	1.45	1.39, 1.51	8,945
	2003-2004	1.43	1.36, 1.50	8,373
	2005-2006	1.29	1.23, 1.36	8,407
	2007-2008	1.27	1.21, 1.34	8,266
	2009-2010	1.12	1.08, 1.16	8,793
1-5 yr	1999-2000	2.23	1.96, 2.53	723
	2001-2002	1.70	1.55, 1.87	898
	2003-2004	1.77	1.60, 1.95	911
	2005-2006	1.46	1.36, 1.57	968
	2007-2008	1.51	1.37, 1.66	817
	2009-2010	1.17	1.08, 1.26	836
6-11 yr	1999-2000	1.51	1.36, 1.66	905
	2001-2002	1.25	1.14, 1.36	1,044
	2003-2004	1.25	1.12, 1.39	856
	2005-2006	1.02	0.95, 1.01	934
	2007-2008	0.99	0.91, 1.07	1,011
	2009-2010	0.84	0.79, 0.89	1,009
12-19 yr	1999-2000	1.10	1.04, 1.17	2,135
	2001-2002	0.94	0.90, 0.99	2,231
	2003-2004	0.95	0.88, 1.02	2,081
	2005-2006	0.80	0.75, 0.85	1,996
	2007-2008	0.80	0.74, 0.86	1,074
	2009-2010	0.68	0.64, 0.73	1,183
≥ 20 yr	1999-2000	1.75	1.68, 1.81	4,207
	2001-2002	1.56	1.49, 1.62	4,772
	2003-2004	1.52	1.45, 1.60	4,525
	2005-2006	1.41	1.34, 1.48	4,509
	2007-2008	1.38	1.31, 1.46	5,364
	2009-2010	1.23	1.19, 1.28	5,765

Table 3-8 (Continued): Blood Pb concentrations in the U.S. population.

Survey Stratum	Period	Geometric Mean (µg/dL)	95% Confidence Interval	Number of Subjects
Males	1999-2000	2.01	1.93, 2.09	3,913
	2001-2002	1.78	1.71, 1.86	4,339
	2003-2004	1.69	1.62, 1.75	4,132
	2005-2006	1.52	1.42, 1.62	4,092
	2007-2008	1.47	1.39, 1.56	4,147
	2009-2010	1.31	1.25, 1.36	4,366
Females	1999-2000	1.37	1.32, 1.43	4,057
	2001-2002	1.19	1.14, 1.25	4,606
	2003-2004	1.22	1.14, 1.31	4,241
	2005-2006	1.11	1.05, 1.17	4,315
	2007-2008	1.11	1.06, 1.16	4,119
	2009-2010	0.97	0.93, 1.01	4,427
Mexican – Americans	1999-2000	1.83	1.75, 1.91	2,742
	2001-2002	1.46	1.34, 1.60	2,268
	2003-2004	1.55	1.43, 1.69	2,085
	2005-2006	1.29	1.21, 1.38	2,236
	2007-2008	1.25	1.15, 1.36	1,712
	2009-2010	1.14	1.03, 1.28	1,966
Non-Hispanic blacks	1999-2000	1.87	1.75, 2.00	1,842
	2001-2002	1.65	1.52, 1.80	2,219
	2003-2004	1.69	1.52, 1.89	2,293
	2005-2006	1.39	1.26, 1.53	2,193
	2007-2008	1.39	1.30, 1.48	1,746
	2009-2010	1.24	1.18, 1.30	1,593
Non-Hispanic whites	1999-2000	1.62	1.55, 1.69	2,716
	2001-2002	1.43	1.37, 1.48	3,806
	2003-2004	1.37	1.32, 1.43	3,478
	2005-2006	1.28	1.19, 1.37	3,310
	2007-2008	1.24	1.16, 1.33	3,461
	2009-2010	1.10	1.04, 1.16	3,760

Age strata correspond to the NHANES study design.

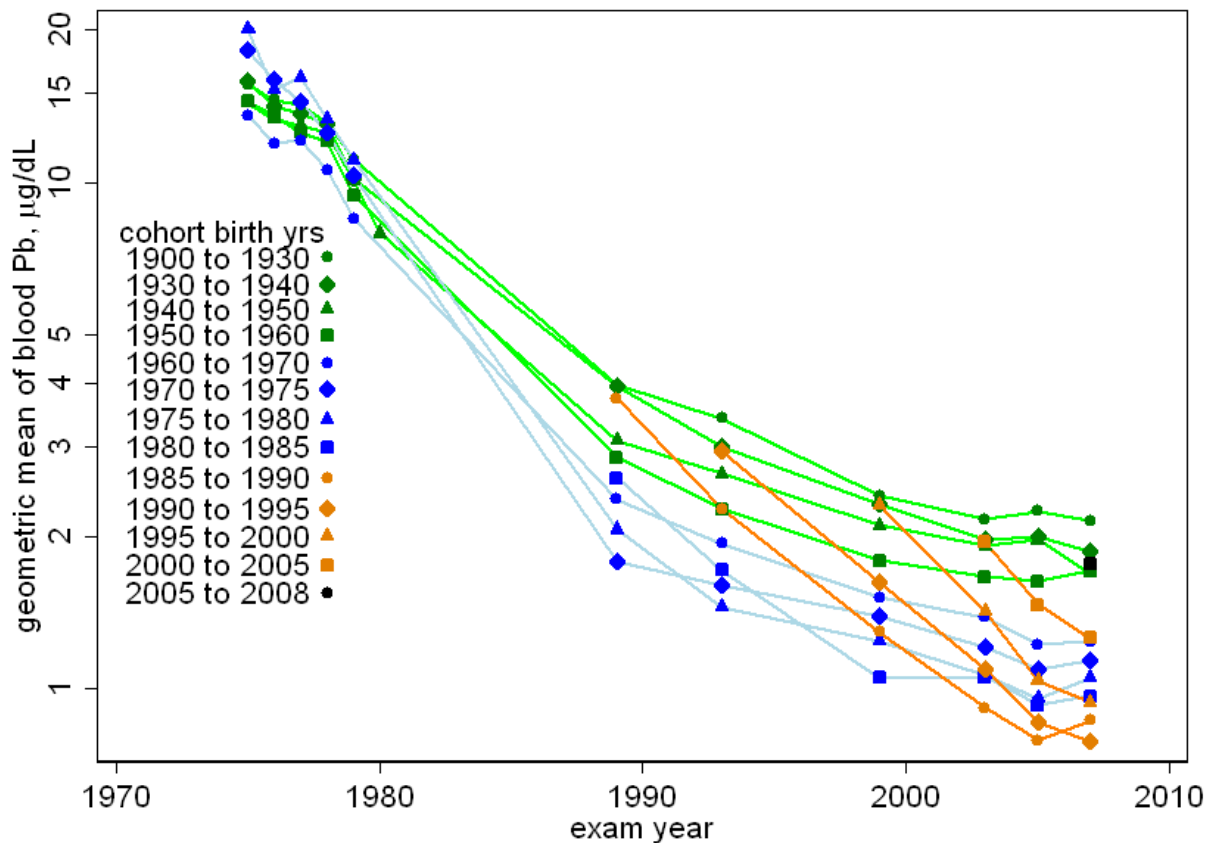
Source: Adapted from data from the NHANES ([CDC, 2013](#)).



Note: Top: all data. Bottom: data for subjects having blood Pb levels less than 15 $\mu\text{g}/\text{dL}$. Dark lines represent the median of the data. Boxes represent the interquartile range of the data, and whiskers represent the 5th-95th percentile range of the data. Outliers beyond the 95th percentile are shown with open circles.

Source: Adapted from data from the NHANES ([NCHS, 2010](https://www.nchs.gov/data/nhanes/nhanes2010/))

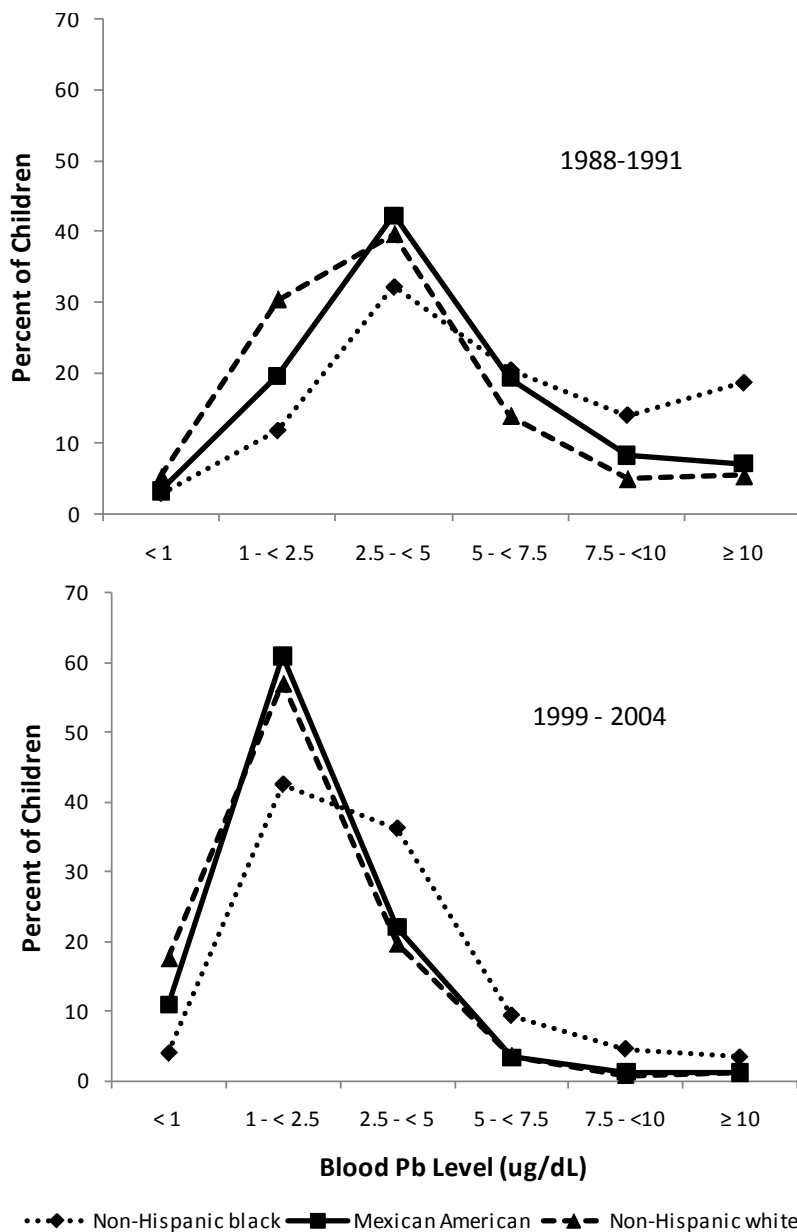
Figure 3-17 Box plots of blood Pb levels among U.S. children (1-5 years old) from the NHANES survey, 1988-2010.



Note: The means of logged blood Pb were weighted to represent national averages. Data were from the publicly available NHANES II, NHANES III for 1988-1991 and 1992-1994, and the continuous NHANES in 1999-2000, 2003-2004, 2005-2006, 2007-2008. Continuous NHANES data from 2001-2002 and 2009-2010 are not included because there were only 551 blood Pb samples in each of those data sets. The year plotted for exam year was the reported exam year for NHANES II, the middle year of each of the phases of NHANES III, and the second year of each of the continuous NHANES.

Source: Adapted from data from the NHANES ([NCHS, 2010](#))

Figure 3-18 Blood Pb cohort means versus year of exam.



Source: Data used with permission of the American Academy of Pediatrics, Jones et al. ([2009a](#))

Figure 3-19 Percent distribution of blood Pb levels by race/ethnicity among U.S. children (1-5 years) from the NHANES survey, 1988-1991 (top) and 1999-2004 (bottom).

In agreement with the 1986 AQCD ([U.S. EPA, 1986a](#)), several studies have shown seasonal variation in blood Pb concentrations in children (e.g., [Havlena et al., 2009](#); [Gulson et al., 2008](#); [Kemp et al., 2007](#); [Laidlaw et al., 2005](#); [Haley and Talbot, 2004](#); [Johnson and Bretsch, 2002](#); [Yiin et al., 2000](#); [Johnson et al., 1996](#)), with elevated concentrations during the warm season and lower levels in the cold season. Seasonal dynamics of blood Pb concentrations in children appear to be caused at least in part by seasonal patterns in access of children to soils and soil properties (e.g., moisture content) that may contribute to seasonal variation in entrainment of soil and dust Pb into breathing zone air ([Laidlaw et al., 2012](#); [Laidlaw et al., 2005](#); [Johnson and Bretsch, 2002](#)). Seasonal variation in blood Pb concentrations occur, and summertime blood Pb concentrations demonstrated the strongest association with soil Pb concentrations ([Johnson and Bretsch, 2002](#)). Laidlaw et al. (2012) observed that air Pb in the PM_{2.5} fraction and PM_{2.5} attributed to soil were elevated in the warm season compared with the cold season. Yiin et al. (2000) also found that geometric mean for blood Pb, floor Pb loading and concentration were statistically significantly higher in the hot season compared with the cold season, although carpet dust Pb loading was statistically significantly higher in the cold season compared with the hot season. However, regression of blood Pb on floor and windowsill dust with and without adjustment for the hot, warm, and cool seasons showed no statistically significant effect of the seasons directly on blood Pb. Meteorological factors appear to contribute to blood Pb seasonality, and the tendency of children to play outside during the warm months may also allow for more contact with soil Pb. Laidlaw et al. (2005) analyzed the temporal relationships between child blood Pb concentrations and various atmospheric variables in three cities (Indianapolis, IN: 1999-2002; Syracuse, NY: 1994-1998; New Orleans, LA: 1998-2003). Blood Pb data was obtained from public health screening programs conducted in the three cities. Blood Pb samples were dominated by children <5 years of age and age distribution varied across the three cities. The temporal variation in blood Pb concentrations in each city was predicted by multivariate regression models that included the following significant variables: PM₁₀, wind speed, air temperature, and soil moisture; as well as dummy variables accounting for temporal displacement of the effects of each independent variable on blood Pb. Laidlaw et al. (2005) reported R² values for the regression models, but did not report the actual regression coefficients. The R² values were as follows: Indianapolis 0.87 (p = 0.004); Syracuse 0.61 (p = 0.0012); New Orleans 0.59 (p <0.00001).

Studies have examined the change in blood Pb with changes in potential Pb sources. Gulson et al. (2004b) observed that children living near a Zn-Pb smelter in Australia had blood Pb levels ranging from 10 to 42 µg/dL, with 55-100% of Pb attributed to the smelter based on isotope ratio analysis. Rubio-Andrade et al. (2011) followed a cohort of 6-8 year old children living within 3.5 km of a Mexican smelter at 0, 6, 12, and 60 months after environmental intervention including removal of 100,000 kg of

Pb-containing dust from roads and homes using high efficiency vacuums. Soil Pb was concurrently obtained but not reported at 6, 12, or 60 months. Median blood Pb level at initiation of the study was 10.1 µg/dL for the 598 initial participants (average age: 7.2 y), and median soil Pb was 3,300 mg/kg at the start of the study. After 60 months, median blood Pb level was 4.4 µg/dL for the remaining 232 participants (average age: 12.2 y), and median soil Pb concentration was 370 mg/kg at that time. Bonnard and McKone (2009) modeled blood Pb of French children ages 21-74 months living within a village containing a Pb smelter and estimated blood Pb levels of 3.2-10.9 µg/dL. Lanphear et al. (1998) noted that the probability of children having blood Pb ≥ 10 µg/dL increases both with exterior soil Pb content and interior Pb dust loading. Mielke et al. (2011b) noted significant increases in percentages of children younger than 7-years old with blood Pb level ≥ 10 µg/dL for those living in inner city New Orleans housing developments (22.9%) compared with children living in communities located on the city outskirts (9.1%). At the same time, median soil Pb was significantly higher in the inner city (438 mg/kg) compared with the city outskirts (117 mg/kg).

For infants <1 year old, very little data are available on blood Pb levels. Simon et al. (2007) followed a cohort of 13 children living near an Australian smelter from birth through 36 months. In general, except for children born with low blood Pb levels of ~ 1 to 2 µg/dL, immediately after birth blood Pb levels fell for 1-2 months to approximately 47% of birth blood Pb level. After this initial fall, all infants' blood Pb levels rose with age until approximately 12 months old for children living in a high risk area and until approximately 18 months for children living in a low risk area (Simon et al., 2007). Median blood Pb level among the children was 1.9 µg/dL at 2 months and increased to 13.6 µg/dL at 16 months. Geometric mean hand-Pb loading of the child and the mother were significant contributors to the area under the curve for infant blood Pb, with 46% (infant hand loading) and 60% (mother hand loading) of the variance being explained by these variables, respectively; geometric mean of the mothers' blood Pb explained 46% of the variance (Simon et al., 2007). Across all the data, there was a good correlation between child blood Pb level and child hand Pb loading ($R^2 = 0.70$). In another study (Carbone et al., 1998), blood Pb levels of 15 infants aged 6-12 months were statistically significantly lower than their neonatal cord blood Pb levels (2.24 µg/dL versus 4.87 µg/dL). Additionally, 3 infants born with blood Pb levels of greater than 7 µg/dL were followed for a week, there was a dramatic drop in the blood Pb of from an average of 7.6 µg/dL on Day 1 to 2.4 µg/dL on Day 7 (Carbone et al., 1998).

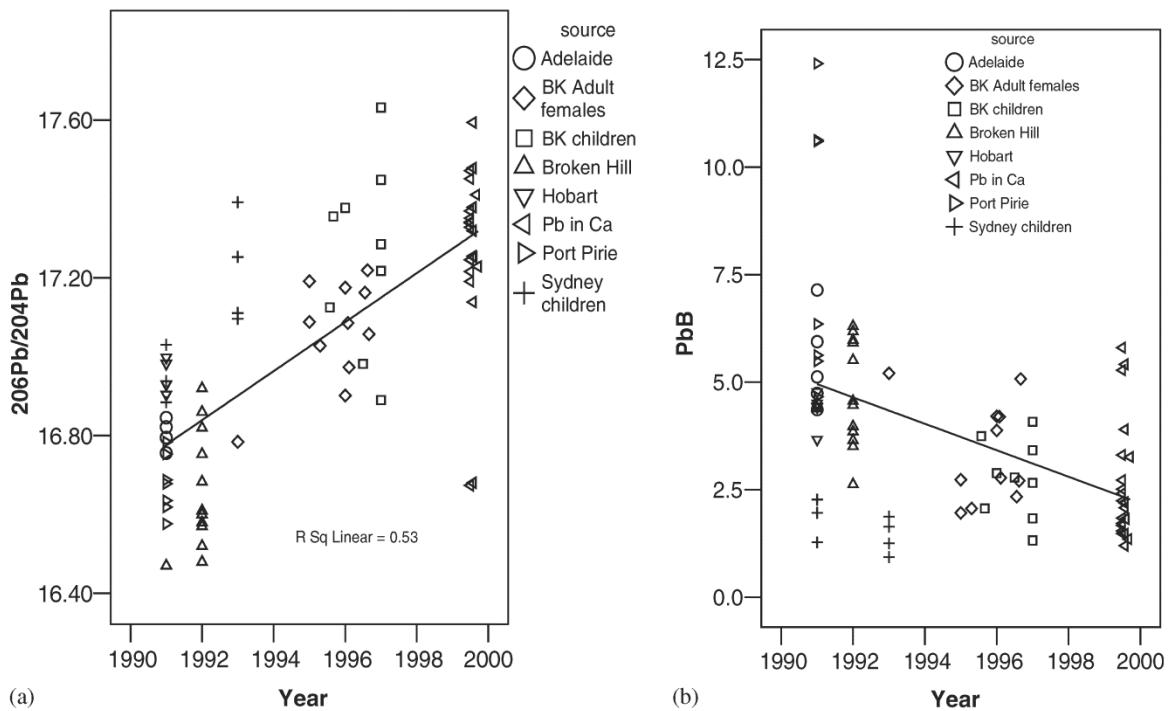
Pb body burden has been reported among individuals known to consume wild game hunted with Pb shot. For example, fifty men from Nuuk, Greenland participated in a study in which they recorded their diet and produced blood samples (Johansen et al., 2006). Men who regularly ate hunted sea birds had an average blood Pb concentration of

12.8 µg/dL, in contrast with those who did not and had an average blood Pb concentration of 1.5 µg/dL. Umbilical cord blood was collected from a cohort of Inuit newborns from northern Quebec, where the Inuit population consumes game killed with Pb shot ([Lévesque et al., 2003](#)). The geometric mean cord blood Pb level was 0.19 µmol/L [3.9 µg/dL], with a range of 0.01-1.31 µmol/L [0.2-27 µg/dL]; the Canadian level of concern for cord blood Pb is 0.48 µmol/L [10 µg/dL]. The authors contrasted the finding that 7% of Inuit newborns had cord blood Pb concentration ≥ 0.48 µmol/L [10 µg/dL] in contrast with 0.16% of the Caucasian population in southern Quebec.

Recent studies have sought to characterize human exposure to Pb from piston-engine aircraft emissions. [Section 2.2.2.1](#) describes a study by Carr et al. ([2011](#)) in which Pb concentrations, both modeled and monitored, extended beyond airport property. Miranda et al. ([2011](#)) used GIS to study the association between blood Pb level and distance from airports in six North Carolina Counties. They observed that the trend in blood Pb level decreases monotonically with distance class from the airports, with subjects within 500 meters of the airports having significantly increased blood Pb levels ($\beta = 0.043$, 95% CI: (0.006, 0.080), $p < 0.05$) compared with the general population for a given county after controlling for proportion of black, Hispanic, percent receiving public assistance, and household median income at the census block group level and including dummy variables for season during which the children were screened for blood Pb. In this study, children living within 500 meters of an airport had blood Pb levels that were, on average, 4.4% higher than those at distance. Note that the authors did not include Pb emissions in their model.

Trends in blood Pb levels have been accompanied by changes in Pb isotope ratios for blood Pb. Isotopic ratios, described in [Sections 2.2](#) and [2.3](#) as a tool for source apportionment, have been used to associate blood Pb measurements with anthropogenic sources of Pb in the environment. Changes in Pb isotopic ratios in blood samples reflect the changing influence of sources of Pb following the phase-out of tetraethyl Pb antiknock agents in automotive gasoline and changes in Pb usage in paints and other industrial and consumer products ([Gulson et al., 2008](#); [Ranft et al., 2008](#); [Gulson et al., 2006a](#); [Ranft et al., 2006](#)). Gulson et al. ([2006a](#)) illustrated how a linear increase in the isotopic ratio $^{206}\text{Pb}/^{204}\text{Pb}$ occurred in concert with a decrease in blood Pb levels among selected study populations in Australia during the period 1990-2000 ([Figure 3-20](#)). Gulson et al. ([2006a](#)) point out that the isotopic signature of $^{206}\text{Pb}/^{204}\text{Pb}$ derived from Australian mines (median ~ 16.8) differs from that of European and Asian mines, where $^{206}\text{Pb}/^{204}\text{Pb}$ varies between ~ 17.4 and ~ 18.1 . Liang et al. ([2010](#)) also examined the trends in blood Pb level over the period 1990 to 2006 in Shanghai and saw a reduction corresponding to the phase out of Pb in gasoline. A plot of $^{208}\text{Pb}/^{206}\text{Pb}$ to $^{207}\text{Pb}/^{206}\text{Pb}$ for blood and environmental samples showed overlap between the isotopic signature for coal

combustion ash and that measured in blood. This result suggests a growing influence of Pb from coal ash in Shanghai in the absence of Pb in automobile emissions. Oulhote et al. (2011) examined Pb isotope ratios in blood Pb samples of 125 French children aged 6 mo-6 years. The study found that Pb isotope ratios could be used to attribute Pb exposure to one source for 32% of children and to eliminate an unlikely source of Pb exposure in 30% of children.



Source: Reprinted with permission of Academic Press, Gulson et al. (2006a).

Figure 3-20 Trends in $^{206}\text{Pb}/^{204}\text{Pb}$ isotope ratio in blood Pb (a) and trends in blood Pb levels (b) among Australian study populations of children during the period 1990-2000.

3.4.2 Pb in Bone

An extensive national database (i.e., NHANES) is available for blood Pb concentrations in children and adults, as described in [Section 3.4.1](#). Bone Pb concentrations are less well characterized. [Table 3-9](#) and [Table 3-10](#) are compilations of data from epidemiologic studies that provided bone Pb concentrations by K-XRF and/or variability in concentrations among individuals without reported occupational exposure and those with occupational exposures, respectively. In non-occupationally exposed individuals, typical group mean tibia bone Pb concentrations ranged from 10 to 30 µg/g. Patella bone Pb levels are typically higher than tibia bone Pb levels in the studies considered ([Table 3-9](#)). For example, in the Normative Aging Study, patella bone Pb concentrations were approximately 32 µg/g, whereas tibia bone Pb concentrations were about 22 µg/g. Occupationally exposed individuals generally had greater bone Pb concentrations than seen in control groups (i.e., unexposed). Bone Pb data in [Table 3-10](#) for occupationally exposed individuals were also generally higher compared to non-occupationally exposed individuals ([Table 3-9](#)).

Table 3-9 Epidemiologic studies that provide bone Pb measurements for non-occupationally exposed populations.

Reference	Study Methods	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Conc. (µg/g)	Distribution of Bone Pb (µg/g)
Bandein-Roche et al. (2009)	Cohort: Baltimore Memory Study cohort Age (yrs): 50-70 N:1,140 Location: Baltimore, MD Study Period: 2001-2005	Cumulative	Tibia	Mean ± SD Tibia: 18.8 ± 11.6	Not reported
Bellinger et al. (1994a)	Cohort: Not reported Age (yrs): 5-8 (recruited); 19-20 (follow-up) N: 79 Location: Boston, MA Study Period: 1989-1990	Cumulative	Tibia Patella	Mean (Range): Tibia: 5.4 (3-16) Patella: 9.2 (4-18)	High exposure: >24 Low exposure: <8.7

Table 3-9 (Continued): Epidemiologic studies that provide bone Pb measurements for non-occupationally exposed populations.

Reference	Study Methods	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Conc. (µg/g)	Distribution of Bone Pb (µg/g)
Cheng et al. (2001)	Cohort: Normative Aging Study cohort Age (yrs): Mean ± SD: Normotensive: 65.49 ± 7.17 Borderline hypertension: 68.3 ± 7.79 Definite hypertension: 67.93 ± 6.79 N: 833 males Location: Boston, MA Study Period: 8/1/1991-12/31/1997	Cumulative	Tibia Patella	Mean ± SD Tibia: Normotensive: 20.27 ± 11.55 Borderline hypertension: 23.46 ± 15.02 Definite hypertension: 22.69 ± 14.71 Patella: Normotensive: 28.95 ± 18.01 Borderline hypertension: 33.73 ± 21.76 Definite hypertension: 32.72 ± 19.55	Lowest quintile: Tibia: 8.5 Patella: 12.0 Highest quintile: Tibia: 36.0 Patella: 53.0
Coon et al. (2006)	Cohort: Participants from Henry Ford Health System (HFHS) Age (yrs): ≥ 50; Mean: 69.9 N: 121 cases; 414 controls Location: Southeastern Michigan Study Period: 1995-1999 (participants received primary health care services)	Cumulative	Tibia Calcaneus	Mean ± SD: Tibia: 12.5 ± 7.8 Calcaneus: 20.5 ± 10.2	Tibia Q1: 0-5.91 Q2: 5.92-10.40 Q3: 10.41-15.50 Q4: ≥ 15.51 Calcaneus Q1: 0-11.70 Q2: 11.71-19.07 Q3: 19.08-25.28 Q4: ≥ 25.29
Elmarsafawy et al. (2006)	Cohort: Normative Aging Study Age (yrs): Not reported N: 471 elderly males Location: Greater Boston area, MA Study Period: 6/1991-12/1994	Not reported	Tibia Patella	Mean± SD: Tibia: 21.6 ±1 2.0 Patella: 31.7 ± 18.3	Not reported

Table 3-9 (Continued): Epidemiologic studies that provide bone Pb measurements for non-occupationally exposed populations.

Reference	Study Methods	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Conc. (µg/g)	Distribution of Bone Pb (µg/g)
Glass et al. (2009)	Cohort: Baltimore Memory Study Age (yrs): Mean: 59.4; Range: 50-70 N: 1,001 Location: Baltimore, MD Study Period: 2001-2005	Cumulative (lifetime)	Tibia	Mean ± SD: Tibia: 18.8 ± 11.1	NPH Scale: Lowest tertile: Mean Tibia level: 16.3 ± 11.0 Middle tertile: Mean Tibia level: 19.3 ± 10.7 Highest tertile: Mean Tibia level: 20.3 ± 11.4
Hsieh et al. (2009b)	Cohort: Not reported Age (yrs): Mean: Control: 46.06 N: 18 controls Location: Not reported Study Period: Not reported	Control group for occupational exposure group	Tibia Patella	Mean ± SD Tibia Control: 18.51 ± 22.40 Patella Control: 7.14 ± 9.81	Not reported
Hu et al. (1996a) [As reported in Navas-Acien et al., (2008)]	Cohort: Normative Aging Study Age (yrs): 48-92; Mean ± SD: 66.6 ± 7.2 N: 590 males Location: Boston, MA Study Period: 8/1991-12/1994	Cumulative	Tibia Patella	Mean ± SD: Tibia: 21.8 ± 12.1 Patella: 32.1 ± 18.7 Range: Tibia: <1-96 Patella: 1-142	Figures 1 and 2 show both types of bone Pb levels increasing with age

Table 3-9 (Continued): Epidemiologic studies that provide bone Pb measurements for non-occupationally exposed populations.

Reference	Study Methods	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Conc. (µg/g)	Distribution of Bone Pb (µg/g)
Jain et al. (2007)	Cohort: VA-Normative Aging Study Age (yrs): Not reported N: 837 males Location: Greater Boston, MA Study Period: 9/1/1991-12/31/2001	Not reported	Tibia Patella	Mean ± SD Tibia: Non-Cases: 21.4 ± 13.6 Cases: 24.2 ± 15.9 Patella: Non-cases: 30.6±19.7 Cases: 36.8 ± 20.8 Range: Tibia: Non-cases: -3-126 Cases: -5-75 Patella: Non-cases: -10-165 Cases: 5-101	Mean ± SD (Range): Tibia: Non-cases: Tertile 1: 10.2 ± 3.8 (-3-15) Tertile 2: 19.1 ± 2.3 (16-23) Tertile 3: 35.5 ± 14.4 (24-126) Cases: Tertile 1: 10.1 ± 5.3 (-5-15) Tertile 2: 19.8 ± 2.2 (16-23) Tertile 3: 39.5 ± 14.9 (25-75) Patella: Non-cases: Tertile 1: 13.9±4.9 (-10-20) Tertile 2: 27.1±4.1 (21-34) Tertile 3: 52.5± 20.7 (35-165) Cases: Tertile 1: 15.3±4.3 (5-19) Tertile 2: 25.7 ± 3.8 (21-33) Tertile 3: 53.3 ± 17.3 (35-101)

Table 3-9 (Continued): Epidemiologic studies that provide bone Pb measurements for non-occupationally exposed populations.

Reference	Study Methods	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Conc. (µg/g)	Distribution of Bone Pb (µg/g)
Kamel et al. (2002); Kamel et al. (2005); Kamel et al. (2008)	Cohort: Not reported Age (yrs): 30-80 N: 256 controls (Bone samples collected from 41 controls) Location: New England (Boston, MA) Study Period: 1993-1996	Cumulative Control group for occupational exposure group	Tibia Patella	Mean ± SE Tibia Controls: 11.1 ± 1.6 Patella Controls: 16.7 ± 2.0	Controls Tibia: N (%) -7-7: 14 (34) 8-14: 12 (29) 15-61: 15 (37) Patella: N (%) -4-9: 14 (34) 10-20: 14 (34) 21-107: 13 (32)
Khalil et al. (2009a)	Cohort: 1982 Lead Occupational Study Age (yrs): Control mean: 55 N: 51 controls Location: Eastern Pennsylvania Study Period: 1982-2004	Control group for occupational exposure group	Tibia	Median (IQR) Tibia Control: 12 (-8-32)	Not reported
Korrick et al. (1999) [As reported in Navas-Acien et al., (2008)]	Cohort: Nurses' Health Study Age (yrs): Combined: 47-74; Mean ± SD: Combined: 58.7 ± 7.2; Cases: 61.1 ± 7.1; High controls: 61.1 ± 7.2; Low controls: 58.7 ± 7.1 N: 284 females; (89 cases; 195 controls) Location: Boston, MA Study Period: 7/1993-7/1995	Nonoccupationally exposed	Tibia Patella	Mean ± SD Tibia: Combined: 13.3 ± 9.0 Cases: 13.0 ± 9.4 High controls: 14.7 ± 10 Low controls: 12.7 ± 8.1 Patella: Combined: 17.3 ± 11.1 Cases: 19.5 ± 12.9 High controls: 17.2 ± 9 Low controls: 15.8 ± 10.6 Range Tibia Combined: -5-69 Patella Combined: -5-87	Patella: 10th percentile: 6 90th percentile: 31

Table 3-9 (Continued): Epidemiologic studies that provide bone Pb measurements for non-occupationally exposed populations.

Reference	Study Methods	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Conc. (µg/g)	Distribution of Bone Pb (µg/g)
Lee et al. (2001a) [As reported in Navas-Acien et al., (2008)]	Cohort: Not reported Age (yrs): 22.0-60.2 Mean ± SD: Controls: 34.5 ± 9.1 N: 135 controls Location: South Korea Study Period: 10/24/1997-8/19/1999	Control group for occupational exposure group	Tibia	Mean ± SD Tibia Controls: 5.8 ± 7.0 Range Tibia Controls: -11-27	Not reported
Martin et al. (2006)	Cohort: Baltimore Memory Study Age (yrs): 50-70; Mean: 59.4 N: 964 Location: Baltimore, MD Study Period: 5/2001-9/2002 (1st study visit) 8/2002-3/2004 (2nd study visit – tibia Pb measured)	Cumulative (lifetime)	Tibia	Mean ± SD Tibia: 18.8 ± 12.4	Tibia IQR: 11.9-24.8
Needleman et al. (2002)	Cohort: Not reported Age (yrs): 12-18; Mean age ± SD: African American cases: 15.8 ± 1.4 African American controls: 15.5 ± 1.1; White cases: 15.7 ± 1.3; White controls: 15.8 ± 1.1 N: 194 male youth cases; 146 male youth controls Location: Allegheny County, PA (cases); Pittsburgh, PA (controls) Study Period: 4/1996-8/1998	Not reported	Tibia	Mean ± SD Tibia Cases (ppm): All subjects: 11.0 ± 32.7 African American: 9.0 ± 33.6 White: 20 ± 27.5 Tibia Controls (ppm): All subjects: 1.5 ± 32.1 African American: -1.4 ± 31.9 White: 3.5 ± 32.6	Table 4 of the paper distributes bone Pb by ≥ 25 or <25 for race, two parental figures, and parent occupation
Osterberg et al. (1997) [As reported in Shih et al., (2007)]	Cohort: Not reported Age (yrs): Median: 41.5 N: 19 male controls Location: Not reported Study Period: Not reported	Control group for occupational exposure group	Finger bone	Median (range) Finger Bone Controls: 4 (-19-18)	Not reported

Table 3-9 (Continued): Epidemiologic studies that provide bone Pb measurements for non-occupationally exposed populations.

Reference	Study Methods	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Conc. (µg/g)	Distribution of Bone Pb (µg/g)
Park et al. (2006)	Cohort: Normative Aging Study Age (yrs): Mean: 72.9 ± 6.5 N: 413 males Location: Greater Boston, MA Study Period: 11/14/2000-12/22/2004; (HRV measurements taken); 1991-2002 (bone Pb measurements taken)	Not reported	Tibia Patella	Median (IQR) Tibia: 19.0 (11-28) Patella: 23.0 (15-34) Estimated Patella ^a 16.3 (10.4-25.8)	Median (IQR) for No. of metabolic abnormalities: Tibia: 0: 18.5 (10.5-23) 1: 19 (11-28) 2: 19 (12-26) Patella: 0: 22 (13.5-32) 1: 25 (16-36) 2: 20 (15-32) Estimated Patella: 0: 16.3 (10.8-24.8) 1: 17.1 (11-29.3) 2: 15.1 (9.4-22.1)
Park et al. (2009a)	Cohort: Normative Aging Study Age (yrs): Mean: 67.3 ± 7.2 N: 613 males Location: Greater Boston, MA Study Period: 8/1991 - 12/1995	Not reported	Tibia Patella	Median (IQR) Tibia: 19 (14-27) Patella: 26 (18-37)	Table 1 of the paper distributes tibia and patella Pb by genotype; Table 2 of the paper distributes tibia and patella Pb by number of gene variants

Table 3-9 (Continued): Epidemiologic studies that provide bone Pb measurements for non-occupationally exposed populations.

Reference	Study Methods	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Conc. (µg/g)	Distribution of Bone Pb (µg/g)
Park et al. (2010)	Cohort: VA Normative Aging Study cohort Age (yrs): Mean: 64.9 (at bone Pb measurement) N: 448 males Location: Eastern Massachusetts Study Period: 1991-1996	Cumulative (chronic exposure)	Tibia Patella	Mean ± SD Tibia: 22.5 ± 14.2 Patella: 32.5 ± 20.4	Tibia IQR: 15 Patella IQR: 21 Table 2 of the paper provides age-adjusted mean bone Pb levels (age, race, education, smoking [pack-yr], occupational noise, noise notch, BMI, hypertension, diabetes)
Payton et al. (1998)	Cohort: VA Normative Aging Study cohort Age (yrs): Mean: 66.8 N: 141 males Location: Boston, MA Study Period: 4/1993-3/1994	Not reported	Tibia Patella	Mean ± SD Tibia: 22.5 ± 12.2 Patella: 31.7 ± 19.2	Not reported
Peters et al. (2007)	Cohort: Normative Aging Study cohort Age (yrs): Mean: 66.9 N: 513 male cases Location: Boston, MA Study Period: 1991-1996	Cumulative	Tibia Patella	Mean ± SD Tibia: 21.5 ± 13.4 Patella: 31.5 ± 19.3	Not reported
Rajan et al. (2007)	Cohort: VA Normative Aging Study Cohort Age (yrs): Mean: 67.5 (at bone scan) N: 1075 males Location: Boston, MA Study Period: 1991-2002	Not reported	Tibia Patella	Mean ± SD Tibia: 22.1 ± 13.8 Patella: 31.4 ± 19.6	Not reported
Rajan et al. (2008)	Cohort: VA Normative Aging Study Cohort Age (yrs): ≥ 45 N: 720 males Location: Boston, MA Study Period: 1993-2001	Current and cumulative	Tibia Patella	Mean ± SD ALAD 1-1 Tibia: 21.9 ± 13.8 Patella: 29.3 ± 19.1 ALAD 1-2/2-2 Tibia: 21.2 ± 11.6 Patella: 27.9 ± 17.3	Not reported

Table 3-9 (Continued): Epidemiologic studies that provide bone Pb measurements for non-occupationally exposed populations.

Reference	Study Methods	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Conc. (µg/g)	Distribution of Bone Pb (µg/g)
Rhodes et al. (2003)	Cohort: VA Normative Aging Study Cohort Age (yrs): Mean: 67.1 N: 526 males Location: Boston, MA Study Period: 1/1/1991-12/31/1995	Not reported	Tibia Patella	Mean ± SD Tibia: 21.9 ± 13.5 Patella: 32.1 ± 19.8	No. of participants (%) Tibia: <1-15: 173 (33) 16-24: 186 (35) 25-126: 167 (32) Patella: <1-22: 189 (36) 23-35: 165 (31) 36-165: 172 (33)
Roels et al. (1994)	Cohort: Not reported Age (yrs): 30-60 N: 68 males Location: Belgium Study Period: Not reported	Control group for occupational exposure group	Tibia	Geometric Mean (Range) Tibia Controls: Normotensive: 21.7 (<15.2-69.3) Hypertensive: 20.2 (<15.2-52.9) Total: 21.4 (<15.2-69.3)	Not reported
Rothenberg et al. (2002a) [as reported in Navas-Acien et al. (2008)]	Cohort: Not reported Age (yrs): 15-44; Mean ± SD: 31.0 ± 7.7 N: 720 females Location: Los Angeles, CA Study Period: 6/1995-5/2001	Not reported	Tibia Calcaneus	Mean ± SD Tibia: 8.0 ± 11.4 Calcaneus: 10.7 ± 11.9	Tibia quartiles: Q1: -33.7-0.9 Q2: 1.0-8.0 Q3: 8.1-16.1 Q4: 16.2-42.5 Calcaneus quartiles: Q1: -30.6-3.0 Q2: 3.1-10.0 Q3: 10.1-18.7 Q4: 18.8-49.0
Shih et al., (2006)	Cohort: Baltimore Memory Study cohort Age (yrs): Mean: 59.39 N: 985 Location: Baltimore, MD Study Period: Not reported	Not reported	Tibia	Mean ± SD: Tibia: 18.7 ± 11.2	Not reported

Table 3-9 (Continued): Epidemiologic studies that provide bone Pb measurements for non-occupationally exposed populations.

Reference	Study Methods	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Conc. (µg/g)	Distribution of Bone Pb (µg/g)
Stokes et al. (1998) [as reported in Shih et al. (2007)]	Cohort: Not reported Age (yrs): 19-29 (in 1994); Mean ± SD: Cases: 24.3 ± 3.18 Control: 24.2 ± 3.02 Cases: 9 months-9 yr (during 1/1/1974-12/31/1975) N: 257 cases; 276 controls Location: Silver Valley, ID; Spokane, WA Study Period: 7/10/1994-8/7/1994	Cumulative (lifelong) Environmental (resided near Pb smelter during childhood)	Tibia	Mean (Range): Tibia Cases: 4.6 (-28.9-37) Tibia Controls: 0.6 (-46.4-17.4)	Tibia No. of Cases: <1 µg/g: 31.5% 1-5 µg/g: 24.4% 5-10 µg/g: 22.3% >10 µg/g: 21.8% No. of Controls: <1 µg/g: 50.4% 1-5 µg/g: 25.6% 5-10 µg/g: 19.4% >10 µg/g: 4.7% Mean ± SD Tibia concentration by age group: Cases: 19-21: 1.47 ± 8.35 22-24: 4.48 ± 7.45 25-27: 4.82 ± 8.92 28-30: 6.64 ± 9.53 Controls: 19-21: 1.27 ± 6.60 22-24: -0.61 ± 6.19 25-27: 0.60 ± 8.60 28-30: 1.74 ± 6.42
Van Wijngaarden et al. (2009)	Cohort: Not reported Age (yrs): Mean: 61.5 N: 47 Location: Rochester, NY Study Period: Not reported	Cumulative	Tibia Calcaneus	Mean ± SD Tibia: 2.0 ± 5.2 Calcaneus: 6.1 ± 8.5	Not reported

Table 3-9 (Continued): Epidemiologic studies that provide bone Pb measurements for non-occupationally exposed populations.

Reference	Study Methods	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Conc. (µg/g)	Distribution of Bone Pb (µg/g)
Wasserman et al. (2003)	Cohort: Yugoslavia Prospective Study of Environmental Pb Exposure Age (yrs): 10-12 N: 167 children Location: Kosovska, Mitrovica, Kosovo, Yugoslavia; Pristina, Kosovo, Yugoslavia Study Period: 5/1985-12/1986 (mother's enrollment); 1986-1999 (follow-up through age 12 yr); Tibia Pb measured 11-13 yr old	Cumulative (lifetime) Environmental (Pb smelter, refinery, battery plant)	Tibia	Mean ± SD: Tibia Pristina: 1.36 ± 6.5 Mitrovica: 39.09 ± 24.55	Tibia quartiles: Q1: -14.4-1.85 Q2: 1.85-10.5 Q3: 10.5-35 Q4: 35-193.5 Table 3 of the paper distributes tibia Pb by sex, ethnicity, address at birth relative to factory, and maternal education
Weisskopf et al. (2004), [as reported in Shih et al. (2007)]	Cohort: Normative Aging Study Age (yrs): Mean ± SD: 67.4 ± 6.6 N: 466 males Location: Boston, MA Study Period: 1991-2002	Environmental	Tibia Patella	Median (IQR) Tibia: 19 (12,26) Patella: 23 (15, 35)	Tibia IQR: 14 Patella IQR: 20 Table 3 of the paper shows mean Pb levels across categorical variables (yr of education, smoking status, computer experience, first language English)
Weisskopf et al. (2007a)	Cohort: VA Normative Aging Study cohort Age (yrs): Mean: Lowest Patella quintile: 73.2; Highest Patella quintile: 80.7 N: 31 males Location: Boston, MA Study Period: Bone Pb measured: 1994-1999 Scans performed: 2002-2004	Not reported	Tibia Patella	Median (IQR) Tibia Lowest quintile: 13 (9-17) Highest quintile: 41 (38-59) Patella Lowest quintile: 9 (5-15) Highest quintile: 63 (43-86)	Not reported

Table 3-9 (Continued): Epidemiologic studies that provide bone Pb measurements for non-occupationally exposed populations.

Reference	Study Methods	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Conc. (µg/g)	Distribution of Bone Pb (µg/g)
Weisskopf et al. (2007b)	Cohort: VA Normative Aging Study cohort Age (yrs): Mean: 68.7 N: 1,089 males Location: Boston, MA Study Period: 1993-2001	Concurrent and cumulative	Tibia Patella	Median (IQR) Tibia: 20 (13-28) Patella: 25 (17-37)	Table 1 of the paper shows distribution of Pb biomarkers by categories of covariates (age, education, smoking status, alcohol intake, physical activity, computer experience, first language English)
Weisskopf et al. (2009)	Cohort: Normative Aging Study; (95% white) Age (yrs): Mean ± SD (at Patella baseline); Tertile 1: 65.2 ± 7.1; Tertile 2: 66.5 ± 6.5 Tertile 3: 70.2 ± 7.2 N: 868 males Location: Greater Boston area, MA Study Period: 1991-1999	Cumulative	Tibia Patella	Mean ± SD Tibia: 21.8 ± 13.6 Patella: 31.2 ± 19.4	Patella tertiles: 1: <22 2: 22-35 3: >35
Weisskopf et al. (2010)	Cohort: BUMC, BWH, BIDMC, HVMA, Normative Aging Study (NAS), Harvard Cooperative Program on Aging (HCPOA) Age (yrs): Mean: Cases: 66.5; Controls: 69.4 N: 330 cases; 308 controls Location: Boston, MA Study Period: 2003-2007 1991-1999 (NAS patients bone Pb measured)	Cumulative	Tibia Patella	Mean ± SD: Tibia: 10.7 ± 12.1 Patella: 13.6 ± 15.9	Tibia quartiles: Q1: <3.1 Q2: 3.5-9.6 Q3: 10.0-17.0 Q4: >17.3 Patella quartiles: Q1: <2.7 Q2: 3.5-11.0 Q3: 11.3-20.9 Q4: >20.9

Table 3-9 (Continued): Epidemiologic studies that provide bone Pb measurements for non-occupationally exposed populations.

Reference	Study Methods	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Conc. (µg/g)	Distribution of Bone Pb (µg/g)
Weuve et al. (2006)	Cohort: VA Normative Aging Study cohort Age (yrs): ≥ 45 N: 720 males Location: Boston, MA Study Period: 1991 (measuring bone Pb levels) End date not reported	Cumulative	Tibia Patella	Median (1st-3rd quartile): Tibia: 19 (13-28) Patella: 27 (18-39)	Table 1 of the paper shows distribution of mean Pb bio-marker levels by characteristics of participants (age, education, computer experience, smoking status, alcohol consumption, tertile of Ca ²⁺ intake, tertile of physical activity, diabetes)
Weuve et al. (2009)	Cohort: Nurses' Health Study cohort Age (yrs): 47-74 N: 587 females Location: Boston, MA Study Period: 1995-2005	Recent and cumulative	Tibia Patella	Mean ± SD: Tibia: 10.5 ± 9.7 Patella: 12.6 ± 11.6	Not reported
Wright et al. (2003) [as reported in Shih et al. (2007)]	Cohort: Normative Aging Study Age (yrs): Mean ± SD: 68.2 ± 6.9 N: 736 males Location: Boston, MA Study Period: 1991-1997	Environmental	Tibia Patella	Mean ± SD: Tibia: 22.4 ± 15.3 Patella: 29.5 ± 21.2	Tibia: Difference in mean from Lowest-highest quartile: 34.2 Patella: Difference in mean from lowest-highest quartile: 47

Table 3-10 Epidemiologic studies that provide bone Pb measurements for occupationally exposed populations.

Reference	Study Methods	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Concentration (µg/g)	Distribution of Bone Pb (µg/g)
Bleecker et al. (1997) [as reported in Shih et al. (2007)]	Cohort: Canada Lead Study Age (yrs): Cumulative: 24-64 Younger: 24-43 Older: 44-64 Mean ± SD: Cumulative: 44.1 ± 8.36 Younger: 37.2 ± 4.57 Older: 50.9 ± 4.86 N: 80 males Location: Canada Study Period: Not Reported	Occupational (Pb smelter workers)	Tibia	Mean ± SD (Tibia): Cumulative: 41.0 ± 24.44 Younger: 35 ± 24.11 Older: 46.9 ± 23.59 Range (Tibia): Cumulative: -12-90 Younger: -12-80 Older: 3-90	Not reported
Bleecker et al. (2007b)	Cohort: Not reported Age (yrs): Mean: 39.7 N: 61 Location: Northern Canada Study Period: Not Reported	Occupational (primary Pb smelter workers)	Tibia	Mean: Tibia: 38.6	Not reported
Caffo et al. (2008)	Cohort: Not reported Age (yrs): Mean: 60.39 N: 513 males Location: Delaware and New Jersey, U.S. Study Period: 1994-1997 (Phase 1 recruitment); 2001-2003 (Phase 2 recruitment)	Cumulative Occupational (Former organolead manufacturing workers)	Tibia	Mean ± SD: Peak Tibia: 23.99 ± 18.46	Not reported
Dorsey et al. (2006)	Cohort: Not reported Age (yrs): Mean: 43.4 N: 652 Location: Korea Study Period: 10/24/1997-8/19/1999 (enrolled)	Occupational (Pb workers)	Tibia Patella	Mean ± SD: Tibia: 33.5 ± 43.4 Patella: 75.1 ± 101.1	Not reported

Table 3-10 (Continued): Epidemiologic studies that provide bone Pb measurements for occupationally exposed populations.

Reference	Study Methods	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Concentration (µg/g)	Distribution of Bone Pb (µg/g)
Glenn et al. (2003) [as reported in Navas-Acien et al. (2008)]	Cohort: Not reported Age (yrs): 40-70; Mean: 55.8 (baseline) N: 496 males Location: Eastern U.S. Study Period: 6/1994-6/1996 (enrolled); 6/1998 (follow-up period ended)	Occupational (Chemical manufacturing facility; inorganic and organic Pb)	Tibia	Mean ± SD: Tibia: 14.7 ± 9.4 (at yr 3) Peak Tibia: 24.3 ± 18.1 Range: Tibia: -1.6-52 (at year 3) Peak Tibia: -2.2-118.8	Not reported
Glenn et al. (2006)	Cohort: Not reported Age (yrs): 0-36.2 (baseline); Mean ± SD: 41.4 ± 9.5 (baseline) N: 575; (76% male; 24% female) Location: South Korea Study Period: 10/1997-6/2001	Cumulative and recent Occupational (Pb-using facilities)	Tibia	Mean ± SD: Tibia: 38.4 ± 42.9 Tibia-Women: Visit 1: 28.2±19.7 Visit 2: 22.8±20.9 Tibia-Men: Visit 1: 41.7±47.6 Visit 2: 37.1±48.1	Not reported
Hanninen et al. (1998) [as reported in Shih et al. (2007)]	Cohort: Not reported Age (yrs): Mean ± SD: Male: 43; Female: 48 Blood Pb (max) ≤ 2.4 µmol/L: 41.7 ± 9.3 Blood Pb (max) >2.4 µmol/L: 46.6 ± 6.2 N: 54; (43 males, 11 females) Location: Helsinki, Finland Study Period: Not reported	Occupational (Pb acid battery factory workers)	Tibia Calcaneus	Mean ± SD: Tibia: Blood Pb (max) ≤ 2.4 µmol/L: 19.8 ± 13.7 Blood Pb (max) >2.4 µmol/L: 35.3 ± 16.6 Calcaneus: Blood Pb (max) ≤ 2.4 µmol/L: 78.6 ± 62.4 Blood Pb (max) >2.4 µmol/L: 100.4 ± 43.1	Not reported

Table 3-10 (Continued): Epidemiologic studies that provide bone Pb measurements for occupationally exposed populations.

Reference	Study Methods	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Concentration (µg/g)	Distribution of Bone Pb (µg/g)
Hsieh et al. (2009b)	Cohort: Not reported Age (yrs): Mean: Cases: 45.71 Controls: 46.06 N: 22 cases; 18 controls Location: Not Reported Study Period: Not reported	Occupational (Pb paint factory workers)	Tibia Patella	Mean ± SD Tibia Case: 61.55 ± 30.21 Control: 18.51 ± 22.40 Patella Case: 66.29 ± 19.48 Control: 7.14 ± 9.81	Not reported
Kamel et al. (2002); Kamel et al. (2005); Kamel et al. (2008)	Cohort: Not reported Age (yrs): 30-80 N: 109 cases; 256 controls; (Bone samples collected from 104 cases and 41 controls) Location: New England (Boston, MA) Study Period: 1993-1996	Cumulative Occupational (Pb fumes, dust, or particles)	Tibia Patella	Mean ± SE Tibia Cases: 14.9 ± 1.6 Controls: 11.1 ± 1.6 Patella Cases: 20.5 ± 2.1 Controls: 16.7 ± 2.0	Cases Tibia Pb: N (%) -7-7: 21 (20) 8-14: 35 (34) 15-61: 48 (46) Patella Pb: N (%) -4-9: 27 (26) 10-20: 40 (38) 21-107: 37 (36) Controls Tibia Pb: N (%) -7-7: 14 (34) 8-14: 12 (29) 15-61: 15 (37) Patella Pb: N (%) -4-9: 14 (34) 10-20: 14 (34) 21-107: 13 (32)
Khalil et al. (2009a)	Cohort: 1982 Pb Occupational Study cohort Age (yrs): Mean: Cases: 54 Controls: 55 N: 83 cases; 51 controls Location: Eastern Pennsylvania Study Period: 1982-2004	Occupational (Pb battery plant workers)	Tibia	Median (IQR) Tibia Cases: 57 (20-86) Controls: 12 (-8-32)	Not reported

Table 3-10 (Continued): Epidemiologic studies that provide bone Pb measurements for occupationally exposed populations.

Reference	Study Methods	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Concentration (µg/g)	Distribution of Bone Pb (µg/g)
Osterberg et al. (1997) [as reported in Shih et al. (2007)]	Cohort: Not reported Age (yrs): Median: 41.5 N: 38 male cases; 19 male controls Location: Not reported Study Period: Not Reported	Occupational (secondary Pb smelter – inorganic Pb)	Finger bone	Median Finger Bone: High Cases: 32 Low cases: 16 Control: 4 Range Finger Bone: High Cases: 17-101 Low cases: -7-49 Control: -19-18	Not reported
Roels et al. (1994)	Cohort: Not reported Age (yrs): 30-60 N: 76 male cases; 68 male controls Location: Belgium Study Period: Not Reported	Occupational (Pb smelter workers) Mean case exposure: 18 yr (range: 6 to 36 yr)	Tibia	Geometric Mean (Range) Tibia Cases: Normotensive: 64.0 (19.6-167.1) Hypertensive: 69.0 (21.7-162.3) Total: 65.8 (19.6-167.1) Tibia Controls: Normotensive: 21.7 (<15.2-69.3) Hypertensive: 20.2 (<15.2-52.9) Total: 21.4 (<15.2-69.3)	Not reported
Schwartz et al. (2000b) [as reported in Shih et al. (2007)]	Cohort: U.S. Organolead Study Age (yrs): Mean ± SD: Cases: 55.6 ± 7.4 Controls: 58.6 ± 7.0 N: 535 male cases 118 male controls Location: Eastern U.S. Study Period: 6/1994-10/1997 (enrolled); Completed 2-4 annual follow-up visits; Tibia Pb taken in 3rd year	Occupational (tetraethyl and tetramethyl Pb manufacturing facility)	Tibia	Mean ± SD <u>Current Tibia:</u> Cases: 14.4 ± 9.3 Peak Tibia: Cases: 22.6 ± 16.5	Not reported

Table 3-10 (Continued): Epidemiologic studies that provide bone Pb measurements for occupationally exposed populations.

Reference	Study Methods	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Concentration (µg/g)	Distribution of Bone Pb (µg/g)
Schwartz et al. (2000c) [as reported in Navas-Acien et al. (2008)]	Cohort: Not reported Age (yrs): 41.7-73.7 (Combined) Mean ± SD: Combined: 57.6 ± 7.6 Hypertensive: 60.2 ± 6.9 Nonhypertensive: 56.6 ± 7.5 N: 543 males Location: Eastern U.S. Study Period: 1995 (recruited); 1996-1997 (Tibia Pb taken during the 3rd yr)	Occupational (former organolead manufacturing workers)	Tibia	Mean ± SD Tibia: Combined: 14.4 ± 9.3 Hypertensive: 15.4 ± 9.1 Nonhypertensive: 14.0 ± 9.3 Range Tibia: Combined: -1.6-52	Not reported
Schwartz et al. (2001); Lee et al. (2001a)	Cohort: Not reported Age (yrs): Mean: Exposed: 40.4 Control: 34.5 N: 803 cases; 135 controls Location: South Korea Study Period: 10/24/1997-8/19/1999	Occupational (battery manufacturing, secondary smelting, Pb oxide manufacturing, car radiator manufacturing)	Tibia	Mean ± SD Tibia Cases: 37.1 ± 40.3 Control: 5.8 ± 7.0 Range: Tibia Cases: -7-338 Controls: -11-27	Not reported
Schwartz et al. (2005)	Cohort: Not reported Age (yrs): Mean at 1st visit: 41.4 N: 576 Location: South Korea Study Period: 10/1997-6/2001	Occupational (current and former Pb workers)	Tibia	Mean ± SD Tibia: 38.4 ± 43	Tibia: 25th percentile at V1: 14.4 75th percentile at V1: 47.1

Table 3-10 (Continued): Epidemiologic studies that provide bone Pb measurements for occupationally exposed populations.

Reference	Study Methods	Prior Pb Exposure	Bone Pb biomarker	Bone Pb Concentration (µg/g)	Distribution of Bone Pb (µg/g)
Stewart et al. (1999) [as reported in Shih et al., (2007)]	Cohort: U.S. Organolead Study Age (yrs): 40-70 (in 1995) 38% ≥ 60 yrs Mean: 58 N: 534 males Location: Eastern U.S. Study Period: Not Reported	Occupational (tetraethyl and tetramethyl Pb manufacturing facility)	Tibia	Mean ± SD Tibia: Current: 14.4 ± 9.3 Peak: 23.7 ± 17.4 Range: Tibia Current: -1.6-52 Peak: -2.2-105.9	Current Tibia Pb: N (%) <5: 77 (14.2) 5-9.99: 113 (20.8) 10-14.99: 119 (21.9) 15-19.99: 117 (21.5) ≥ 20: 118 (21.7) Peak Tibia Pb: N (%) <5: 49 (9.1) 5-9.99: 64 (11.8) 10-14.99: 70 (12.9) 15-19.99: 87 (16.1) 20-24.99: 79 (14.6) 25-29.99: 55 (10.2) ≥ 30: 137 (26.1)
Stewart et al. (2006)	Cohort: Not reported Age (yrs): Mean: 56.1 N: 532 males Location: Eastern U.S. Study Period: 1994-1997; 2001-2003	Cumulative Occupational (Organolead workers - not occupationally exposed to Pb at time of enrollment)	Tibia	Mean ± SD Current Tibia: 14.5 ± 9.6 Peak Tibia: 23.9 ± 18.3	Not reported
Weaver et al. (2008)	Cohort: Not reported Age (yrs): Mean ± SD: 43.3 ± 9.8 N: 652 Location: South Korea Study Period: 12/1999-6/2001	Occupational (Current and former Pb workers; plants produced Pb batteries, Pb oxide, Pb crystal, or radiators, or were secondary Pb smelters)	Patella	Mean ± SD Patella: 37.5 ± 41.8	Not reported

3.4.3 Pb in Urine

Urine Pb concentrations in the U.S. general population have been monitored in the NHANES. Data from the most recent survey ([CDC, 2011a](#)) are shown in [Table 3-11](#). The geometric mean for the entire sample for the period 2007-2008 (N = 2,627) was 0.52 µg/g creatinine (95% CI: 0.48, 0.55). The geometric means for males (N = 1,327) and females (N = 1,300) were 0.50 µg/g creatinine (95% CI: 0.47, 0.53) and 0.53 µg/g creatinine (95% CI: 0.49, 0.57), respectively.

Table 3-11 Urine Pb concentrations in the U.S. population.

Survey Stratum	Period	Geometric Mean (µg Pb/g CR) ^a	95% Confidence Interval	Number of Subjects
All	1999-2000	0.721	0.700, 0.742	2,465
	2001-2002	0.639	0.603, 0.677	2,689
	2003-2004	0.632	0.603, 0.662	2,558
	2005-2006	0.546	0.502, 0.573	2,576
	2007-2008	0.515	0.483, 0.549	2,627
6-11 yr	1999-2000	1.170	0.975, 1.410	340
	2001-2002	0.918	0.841, 1.000	368
	2003-2004	0.926	0.812, 1.060	290
	2005-2006	0.628	0.563, 0.701	355
	2007-2008	0.644	0.543, 0.763	394
12-19 yr	1999-2000	0.496	0.460, 0.535	719
	2001-2002	0.404	0.380, 0.428	762
	2003-2004	0.432	0.404, 0.461	725
	2005-2006	0.363	0.333, 0.395	701
	2007-2008	0.301	0.270, 0.336	376
≥ 20 yr	1999-2000	0.720	0.683, 0.758	1,406
	2001-2002	0.658	0.617, 0.703	1,559
	2003-2004	0.641	0.606, 0.679	1,543
	2005-2006	0.573	0.548, 0.600	1,520
	2007-2008	0.546	0.513, 0.580	1,857
Males	1999-2000	0.720	0.679, 0.763	1,227
	2001-2002	0.639	0.607, 0.673	1,334

Table 3-11 (Continued): Urine Pb concentrations in the U.S. population.

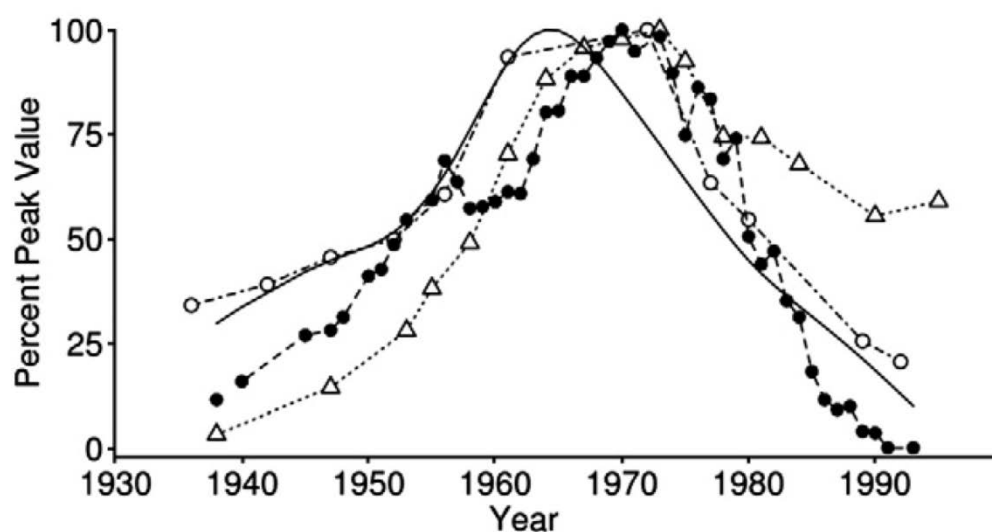
Survey Stratum	Period	Geometric Mean ($\mu\text{g Pb/g CR}$) ^a	95% Confidence Interval	Number of Subjects
Females	2003-2004	0.615	0.588, 0.644	1,281
	2005-2006	0.551	0.522, 0.582	1,271
	2007-2008	0.502	0.471, 0.534	1,327
	1999-2000	0.722	0.681, 0.765	1,238
	2001-2002	0.639	0.594, 0.688	1,355
	2003-2004	0.648	0.601, 0.698	1,277
	2005-2006	0.541	0.507, 0.577	1,305
Mexican - Americans	2007-2008	0.527	0.489, 0.568	1,300
	1999-2000	0.940	0.876, 1.010	884
	2001-2002	0.810	0.731, 0.898	682
	2003-2004	0.755	0.681, 0.838	618
	2005-2006	0.686	0.638, 0.737	652
Non-Hispanic blacks	2007-2008	0.614	0.521, 0.722	515
	1999-2000	0.722	0.659, 0.790	568
	2001-2002	0.644	0.559, 0.742	667
	2003-2004	0.609	0.529, 0.701	723
	2005-2006	0.483	0.459, 0.508	692
Non-Hispanic whites	2007-2008	0.452	0.414, 0.492	589
	1999-2000	0.696	0.668, 0.725	822
	2001-2002	0.615	0.579, 0.654	1,132
	2003-2004	0.623	0.592, 0.655	1,074
	2005-2006	0.541	0.500, 0.585	1,041
	2007-2008	0.506	0.466, 0.550	1,095

^aValues are in $\mu\text{g Pb/g}$ creatinine (CR)

Source: Based on data from the NHANES ([CDC, 2011a](#))

3.4.4 Pb in Teeth

The influence of historical Pb exposures was recently studied by Robbins et al. (2010). Tooth enamel samples from 127 subjects born between 1936 and 1993 were analyzed for Pb concentration and Pb isotope ratios of the tooth enamel and compared with those parameters for sediment cores and estimates of Pb emissions from gasoline during the years when 50% enamel formation was estimated to occur. They found that the log-transform of tooth enamel concentration was significantly predicted by the log-transform of Lake Erie sediment core data obtained by Graney et al. (1995) ($p < 0.00001$) and by the log-transform of U.S. consumption of Pb in gasoline ($p < 0.00001$); see Figure 3-21. Additionally, Robbins et al. (2010) found that $^{207}\text{Pb}/^{206}\text{Pb}$ was significantly predicted by the $^{207}\text{Pb}/^{206}\text{Pb}$ observed in the Lake Erie sediment cores obtained by Graney et al. (1995) ($p < 0.0001$) and for this study ($p < 0.0002$).



Note: The lines and symbols on the plot represent Pb in study participant teeth (solid line), newly obtained Pb sediment Lake Erie cores (open triangles), Pb in previously obtained Lake Erie sediment [open circles, Graney et al. (1995)], and U.S. gasoline usage (closed circles). All values are normalized by the peak observation for that parameter.

Source: Reprinted with permission of Elsevier Publishing, Robbins et al. (2010).

Figure 3-21 Comparison of relative temporal changes in tooth enamel Pb concentration.

Several Brazilian studies have found increased levels of Pb in teeth in areas where Pb sources are present. For example, Costa de Almeida et al. (2007) reported Pb

concentration in tooth enamel among 4-6 year old kindergarteners in São Paulo, Brazil to be significantly higher for children living near a Pb-acid battery processing plant in the Baruru neighborhood compared with 4-6 year old children in other parts of the city (non-exposed median: 206 mg/kg, N = 247; exposed median: 786 mg/kg, N = 26; $p < 0.0001$). Subsequent analysis revealed that 55% of 4-6 year old children from Baruru had tooth enamel Pb concentrations greater than 600 mg/kg, forming a significant comparison with other neighborhoods having 0-33% of 4-6 year old children with tooth enamel Pb greater than 600 mg/kg ($p < 0.0001$) ([de Almeida et al., 2008](#)). The authors did not describe controlling for additional factors, such as socioeconomic or housing conditions. Arruda-Neto et al. ([2009](#)) studied Pb in tooth samples among São Paulo children to compare exposures of children age 4-12 years, living near a dam with heavy metal sediments with those of children ages 4-13 years, living in a control area thought to have few exposures. They observed a significant comparison (near dam: avg 1.28 ± 0.11 mg/kg, N = 50; control region: avg 0.91 mg/kg, N = 24). In a related study of Pb measures in teeth among the general population ages 7-60 years, Arruda-Neto et al. ([2010](#)) observed that 10-year old children had the highest teeth Pb concentrations, which were 115% of the teeth Pb concentrations in 7-year olds. Twenty-year old subjects had teeth Pb concentrations at roughly 50% of the 7-year olds' teeth Pb concentrations. Tooth Pb concentrations stayed fairly constant throughout adulthood but then dropped to just above 30% among 65-year old subjects. Note that the authors did not clarify if average or median values were presented, nor did they adjust for potentially confounding factors.

3.5 Empirical Models of Pb Exposure-Blood Pb Relationships

Multivariate regression models, commonly used in epidemiology, provide estimates of the contribution of variance in the internal dose metric to various determinants or control variables (e.g., air Pb concentration, surface dust Pb concentration). Structural equation modeling links several regression models together to estimate the influence of determinants on the internal dose metric. Regression models can provide estimates of the rate of change of blood or bone Pb concentration in response to an incremental change in exposure level (i.e., slope factor). One strength of regression models for this purpose is that they are empirically verified within the domain of observation and have quantitative estimates of uncertainty imbedded in the model structure. However, regression models are based on (and require) paired predictor-outcome data, and, therefore, the resulting predictions are confined to the domain of observations and are typically not generalizable to other populations. Regression models also frequently exclude numerous parameters that are known to influence human Pb exposures (e.g., soil and dust ingestion rates) and

the relationship between human exposure and tissue Pb levels, parameters which are expected to vary spatially and temporally. Thus, extrapolation of regression models to other spatial or temporal contexts can be problematic.

A variety of factors may potentially affect estimates of blood Pb-air Pb slope factors. Simultaneous changes in other (non-air) sources of Pb exposure can affect the relationship indicated for air Pb. For example, remedial programs (e.g., community and home-based dust control and education) may be responsible for partial blood Pb reduction seen in some studies. The effect of remedial programs may lead to an overestimation of declines in blood Pb due to changes in air Pb and a corresponding positive bias in blood Pb-air Pb slopes. However, model adjustment for remedial programs and other factors (e.g., soil Pb concentrations) may also cause a negative bias in blood Pb-air Pb slopes. A tendency over time for children with lower blood Pb levels to not return for follow-up testing has been reported. The follow-up of children with higher blood Pb levels would likely lead to an underestimation of reductions in blood Pb following reductions in air Pb and cause a negative bias in blood Pb-air Pb slopes. Another factor is the extent to which all the air Pb exposure pathways are captured by the data set and its analysis. For example, some pathways (such as exposure through the diet or surface soils) may respond more slowly to changes in air Pb than others (such as inhalation). Additionally, some studies may include adjustments for variables that also reflect an influence from air Pb (e.g., SES or soil Pb). Studies may also vary in the ages of subjects, which given age-related changes in blood Pb can also influence estimates. Many studies have utilized TSP measurements of air Pb concentrations. The sampling efficiency of TSP samplers is affected by particle size distribution, wind speed, and wind direction as described in [Section 2.4.1](#). For example, especially for larger particles (aerodynamic diameter of 20 μm or more), TSP sampling efficiency decreases with increasing wind speed. Such effects on TSP sampling efficiency can, in areas where such large particles are a substantial portion of airborne Pb, lead to uncertainties in the comparability of air Pb concentrations between samples within a study and across studies. A uniformly low bias in air Pb concentrations in a study could positively bias estimated blood Pb-air Pb slopes for that study. Moreover, variability in TSP samples is likely to result from temporal variation in wind speed, wind direction, and source strength; see [Sections 2.3](#) and [2.5](#). Such temporal variability would tend to increase uncertainty and reduce the statistical strength of the relationship between air Pb and blood Pb but may not necessarily affect the slope of this relationship. A number of factors including those described above cause uncertainty in the magnitude of estimated blood Pb-air Pb slope factors and may lead to both positive and negative biases in the estimates from individual studies.

3.5.1 Air Pb-Blood Pb Relationships in Children

The 1986 Pb AQCD ([U.S. EPA, 1986a](#)) described epidemiological studies of relationships between air Pb and blood Pb. Of the studies examined, the aggregate blood Pb-air Pb slope factor (when considering both air Pb and Pb in other media derived from air Pb) was estimated to be approximately double the slope estimated from the contribution due to inhaled air alone ([U.S. EPA, 1986a](#)).

Much of the pertinent earlier literature (e.g., prior to 1984) on children's blood Pb levels was summarized by Brunekreef ([1984](#)). Based on meta-analysis of data from studies of urban or industrial-urban populations in 18 different locations, Brunekreef ([1984](#)) estimated the blood Pb-air Pb slope for children to be $0.3485 \ln[\mu\text{g/dL blood Pb}]$ per $\ln[\mu\text{g/m}^3 \text{ air Pb}]$ ($R^2 = 0.69$; see [Figure 3-22](#)). This slope corresponds to an increase of $4.6 \mu\text{g/dL blood Pb}$ per $\mu\text{g/m}^3 \text{ air Pb}$ at an air Pb concentration of $1.5 \mu\text{g/m}^3$ for all groups included in the analysis. The $1.5 \mu\text{g/m}^3$ value is the median of the air Pb concentrations that match the blood Pb concentrations in 96 different child populations in Figure 3 of Brunekreef et al. ([1984](#)), taken from the Appendix to the same paper. When the analysis was limited to child populations whose mean blood Pb concentrations were $<20 \mu\text{g/dL}$ ($N=43$), the slope was 0.2159 ($R^2=0.33$), which corresponds to an increase of $4.8 \mu\text{g/dL blood Pb}$ per $\mu\text{g/m}^3 \text{ air Pb}$ at the median air concentration ($0.54 \mu\text{g/m}^3$).

Newer studies that provide estimates for the blood Pb-air Pb slope factor are described in the sections that follow. Those studies that have at least three data points are included, as fewer than that contributes little to the understanding of the shape of the blood Pb-air Pb relationship. A tabular summary of the major outcomes is provided in [Table 3-12](#). In some studies, the blood Pb-air Pb relationship was described with a nonlinear regression function, in which the blood Pb-air Pb slope factor varied with air Pb concentration. Studies also varied with regard to the use of simple or multivariate regression and, for the latter, with regard to variables included. In [Table 3-12](#), with the exception of Ranft et al. ([2008](#)), slopes corresponding to a central estimate of the air Pb concentrations are provided, to represent each study. These were calculated by evaluating each regression function at $\pm 0.01 \mu\text{g/m}^3$ from the central estimate of the air Pb concentration. Air Pb concentration ranges and central estimates varied across studies, making it difficult to interpret comparisons based solely on the central estimates of the slope factors. Therefore, [Figure 3-23](#) depicts the relationship between the blood Pb-air Pb slope factor as a function of air Pb concentration for the range of air Pb concentrations evaluated in those studies that provided the regression equation (the central estimate is also shown). [Figure 3-23](#) provides a more informative picture of the extent to which slope estimates vary (and overlap) within and between studies. The Ranft et al. ([2008](#)) study includes a separate term for soil Pb, so the blood Pb-air Pb slope factor presented for that study

underestimates the slope factor that would reflect all air-related pathways, since soil Pb encompasses deposited ambient air Pb. A few studies used a log-log model that predict an increase in the blood Pb-air Pb slope factor with decreasing air Pb concentration, and the remainder of the studies used linear models that predict a constant blood Pb-air Pb slope factor across all air Pb concentrations.

Table 3-12 Summary of estimated slopes for blood Pb to air Pb slope factors in humans.

Reference	Study Methods	Model Description	Blood Pb– Air Pb Slope ^a
Children Populations – Air			
Brunekreef (1984)	Location: Various countries Years: 1974-1983 Subjects: Children (varying age ranges; N>190,000) Analysis: Meta analysis of 96 child populations from 18 study locations	Model: Log-Log Blood Pb: 5-76 µg/dL (mean range for study populations) Air Pb: 0.1-24 µg/m ³ (mean range for study locations)	All children: 4.6 (1.5) ^b Children <20 µg/dL: 4.8 (0.54) ^c
Hayes et al. (1994)	Location: Chicago, IL Years: 1974-1988 Subjects: 0.5-5 yr (N = 9,604) Analysis: Regression of quarterly median blood Pb and quarterly mean air Pb	Model: Log-Log Blood Pb: 10-28 µg/dL (quarterly median range) Air Pb: 0.05-1.2 µg/m ³ (quarterly mean range)	8.2 (0.62) ^d
Hilts (2003)	Location: Trail, BC Years: 1996-2001 Subjects: 0.5-5 yr, 1996-2000; 0.5-3 yr, 2001 (Estimated N = 220-460 per yr, based on 292-536 eligible children per yr with 75-85% participation) Analysis: Regression of blood Pb screening and community air Pb following upgrading of a local smelter	Model: Linear Blood Pb: 4.7-11.5 µg/dL (annual geometric mean range) Air Pb: 0.03-1.1 µg/m ³ (annual geometric mean range)	7.0 (0.48) ^e
Schwartz and Pitcher (1989), U.S. EPA (1986a)	Location: Chicago, IL Years: 1976-1980 Subjects: Black children, 0-5 yr (N = 5,476) Analysis: Multivariate regression of blood Pb with mass of Pb in gasoline (derived from gasoline consumption data and Pb concentrations in gasoline for the U.S.)	Model: Linear Blood Pb: 18-27 µg/dL (mean range) ^f Air Pb: 0.36-1.22 µg/m ³ (annual maximum quarterly mean) ^h	8.6 (0.75) ^g

Table 3-12 (Continued): Summary of estimated slopes for blood Pb to air Pb slope factors in humans.

Reference	Study Methods	Model Description	Blood Pb– Air Pb Slope ^a
Tripathi et al. (2001)	Location: Mumbai, India (multiple residential locations) Years: 1984-1996 Subjects: 6-10 yr (N = 544) Analysis: Regression of residential location-specific average blood Pb and air Pb data	Model: Linear Blood Pb: 8.6-14.4 µg/dL (GM range for residential locations) Air Pb: 0.10-1.18 µg/m ³ (GM range for residential locations)	3.6 (0.45) ⁱ
Children Populations – Air and Soil^j			
Ranft et al. (2008)	Location: Germany Years: 1983-2000 (blood Pb and air Pb), 2000-2001 (soil Pb) Subjects: 6-11 yr (N = 843) Analysis: Pooled multivariate regression of 5 cross-sectional studies	Model: Log-Linear Blood Pb: 2.2-13.6 µg/dL (5th-95th percentile) Air Pb: 0.03-0.47 µg/m ³ (5th-95th percentile)	3.2, 6.4 ^k
Mixed Child-Adult Populations			
Schwartz and Pitcher (1989), U.S. EPA (1986a)	Location: U.S. Years: 1976-1980 Subjects: NHANES II, 0.5-74 yr, whites (N = 9,987) Analysis: Multivariate regression of blood Pb with mass of Pb in gasoline (derived from gasoline consumption data and Pb concentrations in gasoline for the U.S.)	Model: Linear Blood Pb: 11-18 µg/dL ^g (mean range) ^f Air Pb: 0.36-1.22 µg/m ³ (annual maximum quarterly mean) ^h	9.3 (0.75) ⁱ

^a Slope is predicted change in blood Pb (µg/dL per µg/m³) evaluated at $\pm 0.01 \mu\text{g}/\text{m}^3$ from central estimate of air Pb for the study (shown in parentheses), with the exception of Ranft et al. (2008) in which the slope from the paper is provided because the regression equation was not available. The central estimate for Brunekreef (1984) is the median of air Pb concentrations since it was a meta-analysis; for all other studies the mean is presented. For multiple regression models, this is derived based only on air Pb coefficient and intercept. Depending on extent to which other variables modeled also represent air Pb, this method may underestimate the slope attributable to air pathways. In single regression models, the extent to which non-modeled factors, unrelated to air Pb exposures, exert an impact on blood Pb that covaries with air Pb may lead to the slope presented here to over represent the role of air Pb.

^b $\ln(\text{PbB}) = \ln(\text{PbA}) \times 0.3485 + 2.853$

^c $\ln(\text{PbB}) = \ln(\text{PbA}) \times 0.2159 + 2.620$

^d $\ln(\text{PbB}) = \ln(\text{PbA}) \times 0.24 + 3.17$

^e $\text{PbB} = \text{PbA} \times 7.0$, see Table 3-13 for more information.

^f Observed blood Pb values not provided; data are for regressed adjusted blood Pb.

^g $\text{PbB} = \text{PbA} \times 8.6$

^h Based on air Pb data for U.S. (1986 Pb AQCD) as a surrogate for Chicago.

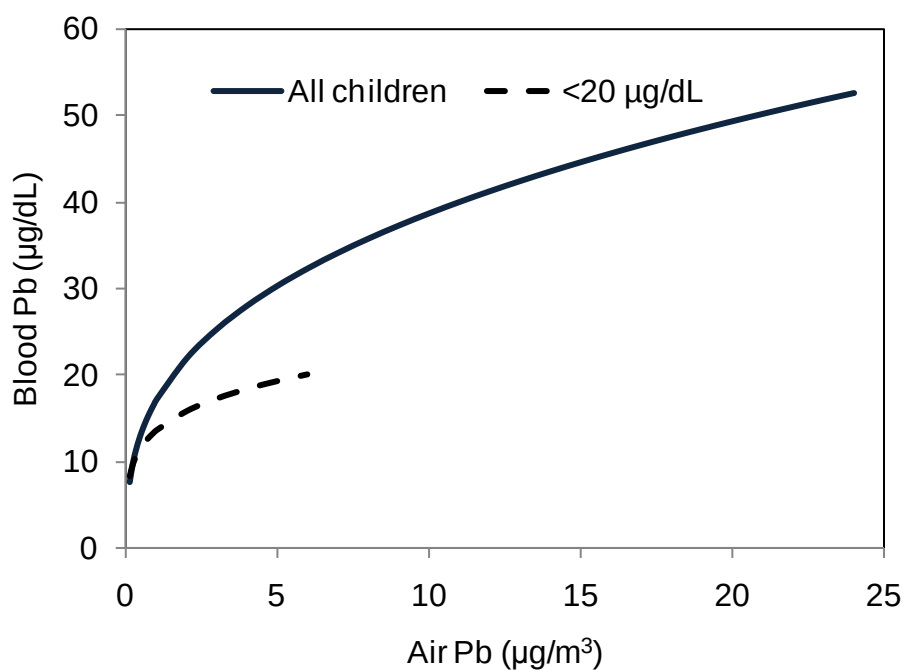
ⁱ $\text{PbB} = \text{PbA} \times 3.6$

^j Study that considered air Pb and soil Pb where the air Pb-blood Pb relationship was adjusted for soil Pb.

^k Slope provided in paper with background blood Pb level of 1.5 and 3 µg/dL, respectively, and GMR of 2.55 for ambient air.

^l $\text{PbB} = \text{PbA} \times 9.63$

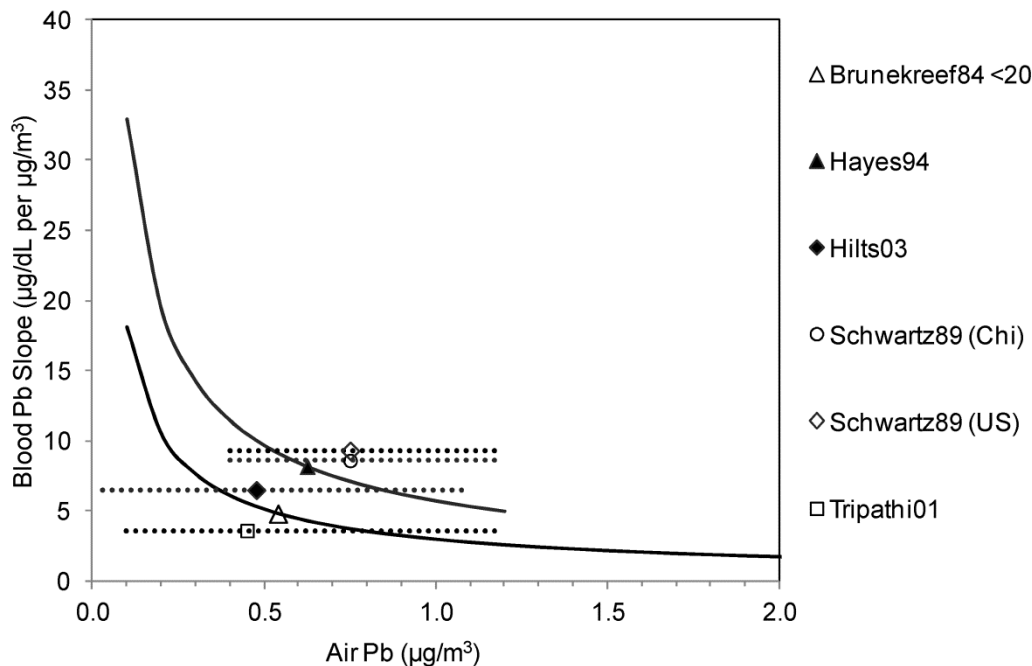
GM, geometric mean; GSD, geometric standard deviation; PbB, blood Pb concentration (µg/dL); PbA, air Pb concentration (µg/m³)



Note: The regression model is: $(\ln[\mu\text{g/dL blood Pb}] = 0.3485 \cdot \ln[\mu\text{g/m}^3 \text{ air Pb}] + 2.85)$ for all children ($n=96$ subject groups) and $(\ln[\mu\text{g/dL blood Pb}] = 0.2159 \cdot \ln[\mu\text{g/m}^3 \text{ air Pb}] + 2.62)$ when the sample was restricted to populations that had blood Pb concentrations $<20 \mu\text{g/dL}$ ($n=44$ subject groups).

Data provided from Brunekreef ([1984](#)).

Figure 3-22 Predicted relationship between air Pb and blood Pb based on a meta-analysis of 18 studies.



Note: Slopes are calculated for a change in air Pb ($\pm 0.01 \mu\text{g}/\text{m}^3$) over ranges of air Pb concentrations reported in each study (lines). The air Pb axis is truncated at $2 \mu\text{g}/\text{m}^3$; the actual range for the Brunekreef et al. (1984) study was $0.1\text{--}6.4 \mu\text{g}/\text{dL per } \mu\text{g}/\text{m}^3$. The slope axis has been truncated at 40; the actual range for the Hayes et al. (1994) study was $5\text{--}56 \mu\text{g}/\text{dL per } \mu\text{g}/\text{m}^3$ (the high end of the range was estimated for the minimum annual average air Pb of $0.05 \mu\text{g}/\text{m}^3$). The two estimates for Schwartz and Pitcher (1989) represent data for U.S. and Chicago. Models are log-log (solid lines) and linear (dotted lines). Symbols show the slope at the central estimate of air Pb (e.g., median for Brunekreef and mean for the other studies).

Figure 3-23 Blood Pb – air Pb slopes ($\mu\text{g}/\text{dL per } \mu\text{g}/\text{m}^3$) predicted from epidemiologic studies.

Hilts (2003) reported child blood Pb and air Pb trends for the city of Trail, British Columbia, over a period preceding and following installation of a new smelter process in 1997 which resulted in lower air Pb concentrations. Blood Pb data were obtained from annual (1989-2001) surveys of children 6-60 months of age who lived within 4 km from the smelter (N: 292-536 eligible per year, 75-85% participation). Air Pb concentrations were obtained from high volume suspended particulate samplers placed within 2 km of the smelter that operated 24 hours every 6th day. Data on Pb levels in air, residential soil, interior dust, and blood for three sampling periods are summarized in Table 3-13. Based on these data, blood Pb decreased $6.5 \mu\text{g}/\text{dL per } 1 \mu\text{g}/\text{m}^3$ air Pb and by $0.068 \mu\text{g}/\text{dL per mg/kg}$ soil Pb (based on linear regression with air or soil Pb as the sole independent variable) for the entire period from 1996-2001. When considering a 9-month weighted mean of $0.13 \mu\text{g}/\text{m}^3$ for 2001 (3 months when the smelter was closed, $0.03 \mu\text{g}/\text{m}^3$; 6 months when it was open, $0.18 \mu\text{g}/\text{m}^3$), the slope is 7.0. Several uncertainties apply to

these estimates. Potential mismatching of air Pb concentrations (often termed misclassification) with individual blood Pb levels may have occurred as a result of air Pb being measured within 2 km of the smelter, whereas, the blood Pb data included children who resided >2 km from the smelter. The regression estimates were based on group mean estimates for three sampling dates, rather than on the individual blood Pb estimates, which included repeated measures on an unreported fraction of the sample. The limited number of data pairs (three) constrained parameter estimates to simple regression coefficients. Other important factors probably contributed to blood Pb declines in this population that may have been correlated with air, soil and dust Pb levels. These factors include aggressive public education and exposure intervention programs ([Hilts et al., 1998](#); [Hilts, 1996](#)). Therefore, the coefficients shown in [Table 3-13](#) are likely to overestimate the influence of air, dust, or soil Pb on blood Pb concentrations at this site.

Table 3-13 Environmental Pb levels and blood Pb levels in children in Trail, British Columbia.

Date	1996 ^a	1999	2001	Regression Coefficient ^d
Age (yrs)	0.5 -5	0.5 -5	0.5 -3	—
Blood Pb (µg/dL)	11.5	5.9	4.7	—
Air Pb (µg/m ³)	1.1	0.3	0.13 ^b	7.01 ± 0.009 (R ² =1.00, p=0.001)
Soil Pb (mg/kg)	844	756	750 ^c	0.069 ± 0.008 (R ² =0.99, p=0.069)
Interior Dust Pb (mg/kg)	758	583	580 ^c	0.035 ± 0.005 (R ² =0.98, p=0.097)

A new smelter process began operation in 1997. Values for air, soil and dust Pb are annual geometric means; values for blood Pb are annual geometric means. Regression coefficients are for simple linear regression of each exposure variable on blood Pb.

^a Values for air Pb, soil Pb, and interior dust Pb are actually for period of 1994-1996.

^b Nine month time-weighted average of 0.03 µg Pb/m³ for 3 months and 0.18 µg Pb/m³ for 6 months.

^c Values assumed by study authors.

^d Slope for change in blood Pb due to change in air, soil, and dust concentrations .

Source: Data from Hilts ([2003](#)).

Ranft et al. ([2008](#)) reported a meta-analysis of five cross-sectional surveys of air and soil Pb levels and blood Pb concentrations in children living in Duisburg, Germany. The analysis included observations on 843 children (6-11 years of age) made during the period 1983-2000. Children recruited in 1983 were an average of 9.1 yrs of age, whereas children recruited in later years of the study averaged 6.3 to 6.4 yrs of age. The 1983 air Pb concentrations were based on two monitoring stations, while a combination of dispersion modeling and monitoring data was used in the later years to estimate Pb in PM in a 200 meter by 200 meter grid that encompassed the city. Pb in surface soil (0-10 cm) was measured at 145 locations in the city in 2000 and 2001. Air and soil Pb concentrations were assigned to each participant by spatial interpolation from the

sampling grid data to each home residence. The 5th-95th percentile ranges were 0.025-0.465 $\mu\text{g Pb/m}^3$ for air and 72-877 mg Pb/kg for soil. The results of multivariate regression analyses were reported in terms of the relative increase (the geometric mean blood Pb ratio, GMR) for an increase in air or soil Pb from the 5th to the 95th percentile value. In a multivariate regression model (equation not provided) that included air and soil Pb in the same model and adjusted for covariates, the GMR values were: 2.55 per 0.44 $\mu\text{g/m}^3$ increase in air Pb (95% CI: 2.40, 2.71, $R^2=0.484$, $p < 0.001$) and 1.30 per 800 mg/kg soil Pb (95% CI: 1.19, 1.43, $R^2 = 0.017$, $p < 0.001$). Based on the values for R^2 , the regression model accounted for approximately 59% of the total variance in blood Pb and, of this, 83% was attributed to air Pb. Values for GMR for soil Pb ranged from 1.41 to 2.89, with most recent blood Pb data (from the year 2000) yielding a value of 1.63 per 800 mg/kg increase in soil Pb. The GMR values can be converted to regression slopes (slope = $[\text{starting blood Pb} \times \ln(\text{GMR})] / [95\text{th} - 5\text{th percentile air or soil Pb}]$) for calculating equivalent air: blood Pb ratios. The model predicts an increase of 3.2 $\mu\text{g/dL}$ blood Pb per 1 $\mu\text{g/m}^3$ increase in air Pb at the median air Pb concentration for the study (0.1 $\mu\text{g/m}^3$) and assuming a background blood Pb concentration of 1.5 $\mu\text{g/dL}$. Based on the GMR estimate of 1.63 for soil Pb, a 1,000 mg/kg increase in soil Pb would be associated with an increase in blood Pb of 0.9 $\mu\text{g/dL}$ per mg/kg soil at the median soil Pb concentration of 206 mg/kg and assuming a background blood Pb concentration of 1.5 $\mu\text{g/dL}$. The degree of confounding of the GMR and estimates resulting from the air and soil Pb correlation was not reported, although the correlation coefficient for the two variables was 0.136 for the whole data set and 0.703 when data collected in 1983 was omitted. Because the model also included Pb levels in soil, the blood Pb-air Pb ratio may be underestimated since some of the Pb in soil was likely derived from air. The blood Pb-air Pb slope does not include the portion of the soil/dust Pb ingestion pathway that derives from air Pb, such as recently airborne Pb deposited to soil and dust which remains available for inhalation and ingestion.

To estimate the blood Pb-air Pb ratio that included all air-related pathways, data for median of blood Pb and air Pb among the cohort of children studied were extracted from Table 2 in Ranft et al. (2008) for each of the five study years. The median blood Pb and air Pb were used in regressions employing linear, log-log, and log-linear (i.e., similar to authors' approach with $\ln[\text{blood Pb}]$ against air Pb) fits. The linear model obtained was: $\text{PbB} = 12.2 \times \text{PbA} + 3.0$ ($R^2 = 0.96$); i.e., the linear regression produced a constant slope of 12 $\mu\text{g/dL}$ per $\mu\text{g/m}^3$. The log-log model was: $\ln(\text{PbB}) = 0.36 \times \ln(\text{PbA}) + 2.4$ ($R^2 = 0.90$), resulting in an inverse curve for $d\text{PbB}/d\text{PbA}$, versus PbA with a slope of 17 $\mu\text{g/dL}$ per $\mu\text{g/m}^3$ at $\text{PbA} = 0.1 \mu\text{g/m}^3$. The log-linear model was: $\ln(\text{PbB}) = 2.2 \times \text{PbA} + 1.2$ ($R^2 = 0.90$), resulting in an exponential curve of $d\text{PbB}/d\text{PbA}$, versus PbA, with a slope of 8.7 $\mu\text{g/dL}$ per $\mu\text{g/m}^3$ at $\text{PbA} = 0.1 \mu\text{g/m}^3$. Geometric mean data for blood Pb and air Pb

are also available from Figure 1 in Ranft et al. (2008) and provide similar regression coefficients and R^2 values to those described above using median values.

Schnaas et al. (2004) analyzed data on blood Pb and air Pb concentrations during and after the phase out of leaded gasoline use in Mexico (1986-1997) in children as part of a prospective study conducted in Mexico City. The sample included 321 children born during the period 1987 through 1992. Repeated blood Pb measurements were made on each child at 6-month intervals up to age 10 years. Air Pb measurements (annual average of quarterly means) were derived from three area monitors which represented distinct study zones. Children were assigned to study zones based on their current address. Associations between lifetime (across the first 10 years of life) blood Pb concentration, air Pb concentration (mean annual for each calendar year of study) and other variables (e.g., age, year of birth, family use of glazed pottery) were evaluated using multivariate regression models. The largest air Pb coefficient occurred in the cohort born in 1987, who experienced the largest decline in air Pb (from 2.8 to about 0.25 $\mu\text{g}/\text{m}^3$); the air Pb coefficient for this group of children was 0.213 (95% CI: 0.114-0.312) $\ln [\mu\text{g}/\text{dL blood}]$ per $\ln [\mu\text{g}/\text{m}^3 \text{ air}]$. The smallest, statistically significant air Pb coefficient occurred for the 1990 birth year cohort, who experienced a decline in air Pb from 1.5 to about 0.1 $\mu\text{g}/\text{m}^3$. The air Pb coefficient for the 1990 cohort was 0.116 (95% CI: 0.035-0.196). Based on these air Pb coefficients, children in the 1987 and 1990 cohorts were estimated to have 24% and 12% decreases in lifetime (across the first 10 years of life) blood Pb levels, respectively, per natural log decrease in air Pb. Table 3-14 provides predicted blood Pb and blood Pb-air Pb slopes as a function of age for the 1987 and 1990 cohorts. The values in Table 3-14 are for children having complete datasets that lived in the Merced (study region having medium air Pb concentrations) of Mexico City in medium SES families (for the study population) that did not use clay pottery. Higher estimated blood Pb and blood Pb-air Pb slopes than those in Table 3-14, would be predicted for low SES families living in Xalostoc (study region having highest air Pb) that use clay pottery (e.g., 2-year-olds predicted blood Pb of 11–14 $\mu\text{g}/\text{dL}$ and blood Pb-air Pb slope of 5.7–7.2 $\mu\text{g}/\text{dL}$ per $\mu\text{g}/\text{m}^3$). Conversely, lower estimates would be predicted for high SES families living in Pedregal (study region having lowest air Pb) that did not use clay pottery (e.g., 2-year-olds predicted blood Pb of 7–9 $\mu\text{g}/\text{dL}$ and blood Pb-air Pb slope of 3.8–4.8 $\mu\text{g}/\text{dL}$ per $\mu\text{g}/\text{m}^3$). The effect of air Pb on blood Pb may have been underestimated in this study due to inclusion of location and SES terms in the regression model. It was specifically noted by the authors that air Pb differed significantly between the locations and the poorer residential areas were usually the more industrialized areas with higher pollution. Hence, the inclusion of these terms may have accounted for some of the variance in blood Pb attributable to air Pb.

Table 3-14 Predicted blood Pb levels and blood-air slopes for Mexico City children (1987 and 1990 cohorts).

Age (in years)	Blood Pb (µg/dL)	Blood Pb–Air Pb Slope (µg/dL per µg/m ³)
1	7.4 – 8.5 ^a	2.1 – 4.5 ^a
2	8.9 – 10.2	2.6 – 5.5
3	8.4 – 9.7	2.4 – 5.2
4	7.9 – 9.1	2.3 – 4.9
5	7.7 – 8.8	2.2 – 4.7
6	6.9 – 7.9	2.0 – 4.2
7	6.8 – 7.8	2.0 – 4.2
8	6.1 – 7.1	1.8 – 3.8
9	5.8 – 6.7	1.7 – 3.6
10	5.6 – 6.5	1.6 – 3.5

^aValues are for 1990 and 1987 cohorts, respectively, at an air Pb concentration of 0.4 µg/m³ which is the median and geometric mean of the annual air Pb concentrations over the course of the study based on data in Figure 1 of Schnaas et al. (2004)

Source: Based on Table 4 of Schnaas et al. (2004)

For an approach that considers all the Schnaas et al. (2004) cohorts simultaneously, data for annual geometric mean of blood Pb and air Pb were extracted from Figure 1 in Schnaas et al. (2004). However, in employing this approach, blood Pb is confounded by age and year because in the early years of the study, only younger children were available and in the later years of the study, only older children contributed data. The extracted values of the geometric mean of blood Pb and mean air Pb were used in regressions employing linear and log-log models for comparison to other studies. The linear model obtained was: $PbB = 2.50 \times PbA + 5.61$ ($R^2 = 0.84$), i.e., the linear model produced a constant slope of 2.50 µg/dL per µg/m³. However, inspection of the graph (not shown here) suggested a bi-linear fit. Regression of the data over the interval 0.1-0.4 µg/m³ produced a slope of 9.0 µg/dL per µg/m³ ($R^2 = 0.83$), and regression of the data over the interval 0.4-2.8 µg/m³ produced a slope of 1.52 µg/dL per µg/m³ ($R^2 = 0.83$). The log-log model was: $\ln(PbB) = 0.26 \times \ln(PbA) + 2.20$ ($R^2 = 0.94$), resulting in an inverse curve for $dPbB/dPb$, versus PbA , with a slope of 4.5 µg/dL per µg/m³ at $PbA = 0.4$ µg/m³.

Schwartz and Pitcher (1989) reported a multivariate regression analysis of associations between U.S. gasoline Pb consumption (i.e., sales) and blood Pb concentrations in the U.S. population during the period 1976-1980 when use of Pb in gasoline was being phased out. Although this analysis did not directly derive a slope for the air Pb-blood Pb relationships, other analyses have shown a strong correlation between U.S. gasoline Pb consumption and ambient air Pb levels during this same period (U.S. EPA, 1986a).

Therefore, it is possible to infer an air Pb-blood Pb relationship from these data. Two sources of blood Pb data were used in Schwartz and Pitcher (1989): NHANES II provided measurements for U.S. individuals 6 months to 74 years of age (N = 9,987 subjects) between February 1976 and February 1980, and the Chicago blood Pb screening program provided measurements in black children aged birth to 5 years (N = 5476 subjects) for the period from 1976 to mid-1980. Observed blood Pb levels were not provided. Gasoline Pb consumption for the U.S. was estimated as the product of monthly gasoline sales and quarterly estimates of Pb concentrations in gasoline reported to U.S. EPA. Based on the NHANES blood Pb data for whites, the regression coefficient for blood Pb on the previous month's gasoline Pb usage, adjusted for age, race, sex, income, degree of urbanization, nutrient intake, smoking, alcohol consumption, occupational exposure, and other significant covariates was 2.14 µg/dL blood per 100 metric tons of gasoline Pb/day (SE=0.19, p=0.0000); the authors reported that the results for blacks were essentially identical. Based on the Chicago blood Pb data, the age-adjusted regression coefficient was 16.12 (µg/dL per 1,000 metric tons gasoline Pb/quarter [SE=1.37, p=0.0001]). When the coefficient was scaled by the ratio of Chicago's gasoline use to the nation's and converted to units of 100 metric tons per day, the gasoline Pb coefficient was 1.97 µg/dL blood per 100 metric tons of gasoline Pb/day), which is similar to the coefficient reported for the NHANES cohort. U.S. EPA (1986a) reported data on gasoline Pb consumption (sales) and ambient air Pb levels in the U.S. during the period 1976-1984 (Table 3-15). Based on these data, air Pb concentrations decreased in association with gasoline Pb consumption. The linear regression coefficient for the air Pb decrease was 0.23 µg/m³ per 100 metric tons gasoline Pb/day (SE = 0.02, R² = 0.95, p <0.0001). If this regression coefficient is used to convert the blood Pb slopes from Schwartz and Pitcher (1989), the corresponding air Pb-blood Pb slopes would be 9.3 and 8.6 µg/dL per µg/m³, based on the NHANES and Chicago data, respectively (e.g., 2.14/0.23 = 9.3 and 1.97/0.23=8.6).

Table 3-15 U.S. gasoline Pb consumption and air Pb levels.

Date	Total Gasoline Pb (10 ³ metric tons/year)	Total Gasoline Pb (10 ² metric tons/day) ^a	Air Pb (µg/m ³) ^b
1976	171.4	4.70	1.22
1977	168.9	4.63	1.20
1978	153.0	4.19	1.13
1979	129.4	3.53	0.74
1980	78.8	2.16	0.66
1981	60.7	1.66	0.51
1982	59.9	1.64	0.53
1983	52.3	1.43	0.40
1984	46.0	1.26	0.36

The linear regression coefficient is 0.23 µg/m³ air per 100 metric tons/day (SE= 0.020, R² = 0.95, p <0.0001)

^aConversion factor is 10/365 days/year.

^bAnnual mean of per site maximum quarterly means (1984 Trend Report, Available online:

http://www.epa.gov/air/airtrends/pdfs/Trends_Report_1984.pdf

Source: Table 5-5, U.S. EPA 1986 Pb AQCD ([1986a](#)).

Tripathi et al. ([2001](#)) reported child blood Pb and air Pb for the city and suburbs of Mumbai, India over the period 1984-1996. Pb-free petroleum was introduced in India beginning in late 1996, which was outside the period of this study. Blood Pb data were obtained from children 6-10 years of age (N = 544) who lived in 13 locations within the Mumbai area. Air Pb concentrations were measured from high volume PM samplers (with the majority of Pb in the respirable size range) placed at a height of 1.6 meters that operated 24 hours. Data on Pb concentrations in air and blood are summarized in [Table 3-16](#). An additional 16 children from two regions of Mumbai were excluded from the analysis because of their high blood Pb levels (geometric means: 69.2 and 20.8 µg/dL) and proximity to industrial Pb sources with high air Pb concentrations (geometric means: 41.2 and 6.7 µg/m³). Based on the data from residential locations presented in [Table 3-16](#), blood Pb increased 3.6 µg/dL per 1 µg/m³ air Pb (based on linear regression with air Pb as the sole independent variable). Several uncertainties apply to these estimates, including potential exposure misclassification since the mean air Pb concentration was used for each suburb over the entire study period. In addition, the regression estimates were based on group mean blood Pb estimates for the 13 sampling locations, rather than on the individual blood Pb estimates. Ingestion of Pb-containing food was estimated in this study, but was not considered in the regression equation for estimating blood Pb, despite the author's conclusion that the ingestion route is important for the intake of Pb by children in Mumbai.

Table 3-16 Air Pb concentrations and blood Pb levels in children in Mumbai, India.

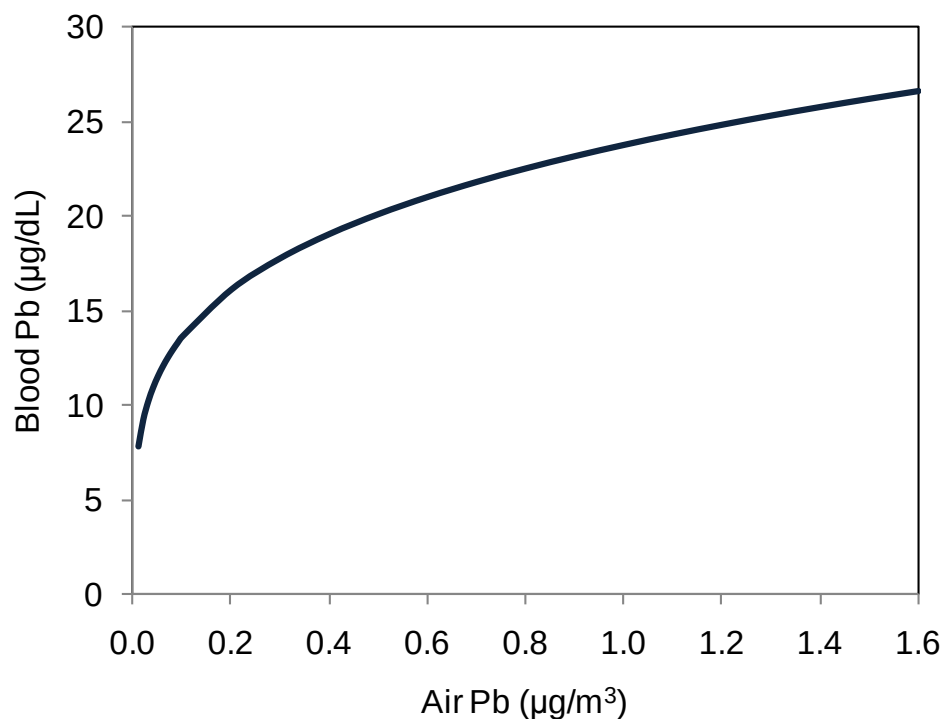
Location	Blood Pb ($\mu\text{g/dL}$)			Air Pb ($\mu\text{g/m}^3$)		
	N	GM	GSD	N	GM	GSD
Borivilli	12	10.4	1.67	10	0.32	1.51
Byculla	117	11.0	1.99	30	0.99	1.73
Deonar	46	9.5	2.29	93	0.11	3.21
Goregaon	21	9.1	1.30	24	0.35	1.77
Govandi	20	8.9	1.42	10	0.10	1.52
Jogeshwari	20	8.6	1.32	24	0.11	2.47
Khar	17	9.0	1.53	22	0.18	3.15
Parel	168	10.4	1.91	37	0.44	1.48
Sion	34	9.6	1.49	96	0.39	1.75
Thans (SS)	37	12.0	1.86	4	1.18	1.04
Vile Parle	19	9.1	1.46	7	0.37	1.34
Colaba	12	9.2	1.86	9	0.14	1.63
Vakola	21	14.4	1.64	7	1.12	1.12

The linear regression coefficient is $3.62 \mu\text{g/dL}$ blood per $\mu\text{g/m}^3$ air ($\text{SE} = 0.61$, $R^2 = 0.76$, $p < 0.001$).

GM, geometric mean; GSD, geometric standard deviation; N, number of subjects.

Source: Data are from Tripathi et al. (2001).

Hayes et al. (1994) analyzed data collected as part of the Chicago, IL blood Pb screening program for the period 1974-1988, following the phase-out of leaded gasoline. The data included 9,604 blood Pb measurements in children (age: 6 months to 5 years) and quarterly average air Pb concentrations measured at 12 monitoring stations in Cook County, IL. Annual median blood Pb levels declined from $30 \mu\text{g/dL}$ in 1968 to $12 \mu\text{g/dL}$ in 1988. During most of the years of the study, blood Pb measurements at or below $10 \mu\text{g/dL}$ were recorded as $10 \mu\text{g/dL}$ because of concerns over measurement accuracy of the instrument below these levels. Quarterly median blood Pb levels declined in association with quarterly mean air Pb concentrations. The regression model predicted a slope of $0.24 \ln [\mu\text{g/dL blood}]$ per $\ln [\mu\text{g/m}^3 \text{ air}]$, as illustrated in Figure 3-24. This slope corresponds to an increase of $8.2 \mu\text{g/dL}$ blood Pb per $\mu\text{g/m}^3$ at the average annual mean air Pb concentration of $0.62 \mu\text{g/m}^3$. As shown in Figure 3-24, with decreasing air Pb concentration, the slope increases. The study reports a slope of 5.6 associated with ambient air Pb levels near $1 \mu\text{g/m}^3$ and a slope of 16 for ambient air Pb levels in the range of $0.25 \mu\text{g/m}^3$, indicating a pattern of higher ratios with lower ambient air Pb and blood Pb levels.



Note: The regression model is: $\ln[\mu\text{g/dL blood Pb}] = 0.24 \cdot \ln[\mu\text{g/m}^3 \text{ air Pb}] + 3.17$.

Modified from Hayes et al. ([1994](#)).

Figure 3-24 Predicted relationship between air Pb and blood Pb based on data from Chicago, IL in children age 0-5 years (1974–1988).

The evidence on the quantitative relationship between air Pb and blood Pb is now, as in the past, limited by the circumstances in which the data are collected. These estimates are generally developed from studies of populations in various Pb exposure circumstances. The 1986 Pb AQCD ([U.S. EPA, 1986a](#)) discussed the studies available at that time that addressed the relationship between air Pb and blood Pb, recognizing that there is significant variability in air-to-blood ratios for different populations exposed to Pb through different air-related exposure pathways and at different exposure levels. The 1986 Pb AQCD noted that ratios derived from studies involving higher blood and air Pb levels are generally smaller than ratios from studies involving lower blood and air Pb levels [see the 1986 Pb AQCD, Chapter 11, pp 99 ([U.S. EPA, 1986a](#))]. In consideration of this factor, slopes in the range of 3 to 5 for children generally reflected study populations with blood Pb levels in the range of approximately 10–30 µg/dL [see Chapter 11, pp 100 of the 1986 Pb AQCD, Table 11-36, from ([Brunekreef, 1984](#))], much higher than those common in today's population. The slope of 3.6 from Tripathi et al.

(2001) is consistent with this observation, given that the blood Pb levels were at the lower end of this range (i.e., 10-15 $\mu\text{g}/\text{dL}$).

There are fewer studies that evaluate the air Pb-blood Pb relationship in conditions that are more reflective of the current state. Hilts (2003) is one such study that provides insight because the blood Pb and air Pb levels were relatively lower than those studies mentioned above; the slope reported was 6.5, but could be as high as 7.0. Similarly, Hayes et al. (1994) demonstrates greater slopes observed with decreasing air Pb concentrations. These studies provide evidence that air-to-blood slopes relevant for today's population of children would likely extend higher than the 3 to 5 range identified in the 1986 Pb AQCD (U.S. EPA, 1986a). Due to the limited evidence, there is increased uncertainty in projecting the magnitude of the air Pb-blood Pb relationship to ambient air Pb concentrations below $0.2 \mu\text{g}/\text{m}^3$. In the 2008 final rule for the Pb NAAQS (73 FR 66964), with recognition of uncertainty and variability in the absolute value of an air-to-blood relationship, the air-to-blood slopes of 5, 7, and $10 \mu\text{g}/\text{dL}$ per $\mu\text{g}/\text{m}^3$ were utilized in evaluating air-related IQ loss of children. Figure 3-25 illustrates the impact of these air-to-blood slopes on the estimated change in air-related blood Pb as a function of change in air Pb.

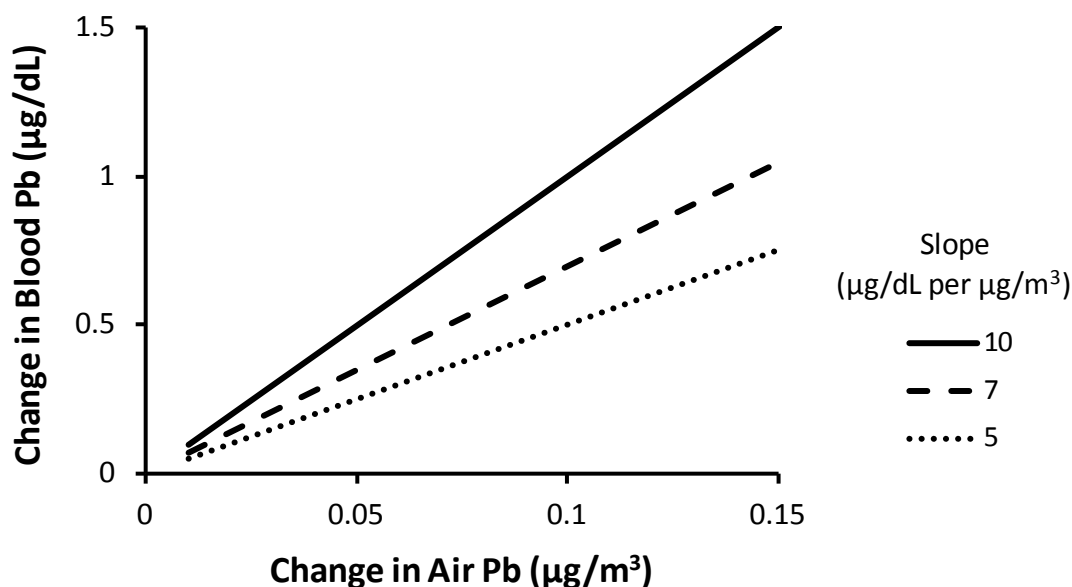


Figure 3-25 Effect of air-to-blood slope on estimated change in air-related blood Pb with change in air Pb.

3.5.2 Air Pb-Blood Pb Relationships in Occupational Cohorts

At the time of the 1986 Pb AQCD, there was a great deal of information on blood Pb responses to air Pb exposures of workers in Pb-related occupations ([U.S. EPA, 1986a](#)). Almost all such exposures were at air Pb exposures far in excess of typical non-occupational exposures and typically did not account for other potential sources of Pb exposure. The air Pb-blood Pb slopes in these studies were generally much less (i.e., 0.03-0.2 $\mu\text{g/dL}$ per $\mu\text{g/m}^3$; pg 11-106) than those observed in children when considering aggregate air Pb contributions (i.e., 3-5 $\mu\text{g/dL}$ per $\mu\text{g/m}^3$; pg 11-106). In addition, the air Pb concentrations in occupational studies are typically collected at much shorter durations (e.g., over an 8-hr workday) compared to ambient air Pb monitoring, making it difficult to draw comparisons between occupationally and non-occupationally exposed populations. Therefore, only a few occupational studies are presented below to demonstrate that more recent air Pb and blood Pb levels remain much higher in these studies compared to those conducted in the general population.

Rodrigues et al. ([2010](#)) examined factors contributing to variability in blood Pb concentration in New England bridge painters, who regularly use electric grinders to prepare surfaces for painting. The study included 84 adults (83 males, 1 female) who were observed during a 2-week period in 1994 or 1995. The geometric mean air Pb concentration obtained from personal PM samplers worn over the workday was 58 $\mu\text{g/m}^3$ (GSD 2.8), with a maximum daily value of 210 $\mu\text{g/m}^3$. Hand wipe samples were collected and analyzed for Pb (GM = 793 μg , GSD 3.7). Blood Pb samples were collected at the beginning of the 2-week period (GM = 16.1 $\mu\text{g/dL}$, GSD 1.7) and at the end of the period (GM=18.2 $\mu\text{g/dL}$, GD=1.6). Associations between exposure variables and blood Pb concentrations were explored with multivariate regression models. When the model excluded hand-wipe data, the regression coefficient for the relationship between $\ln[\text{blood Pb concentration } (\mu\text{g/dL})]$ and $\ln[\text{air Pb } (\mu\text{g/m}^3)]$ was 0.11 (SE = 0.05, $p = 0.03$). This corresponds to a slope of 0.009 $\mu\text{g/dL}$ per $\mu\text{g/m}^3$ at the geometric mean air Pb concentration for the study. A second regression model included hand wipe Pb ($n = 54$) and yielded a regression coefficient of 0.05 (SE = 0.07, $p = 0.45$), which corresponds to a slope of 0.02 $\mu\text{g/dL}$ per $\mu\text{g/m}^3$ at the geometric mean air Pb concentration for the study.

Two other studies that examined the air Pb-blood Pb relationship in occupational settings at higher air Pb concentrations (geometric mean of 82 and 111 $\mu\text{g/m}^3$) for Pb battery and crystal workers, respectively ([Pierre et al., 2002](#); [Lai et al., 1997](#)). Blood Pb levels for the Pb battery workers averaged 56.9 $\mu\text{g/dL}$ (SD 25.3) and for the crystal workers was 21.9 $\mu\text{g/dL}$. Both studies employed log-log regression models, resulting in slopes of 0.49 $\mu\text{g/dL}$ per $\mu\text{g/m}^3$ ([Pierre et al., 2002](#)) and 0.08 $\mu\text{g/dL}$ per $\mu\text{g/m}^3$ ([Lai et al., 1997](#)).

3.5.3 Environmental Pb-Blood Pb Relationships

Empirically-based relationships between blood Pb levels and Pb intakes and/or Pb concentrations in environmental media have provided the basis for what has become known as slope factor models. Slope factor models are highly simplified representations of empirically based regression models in which the slope parameter represents the change in blood Pb concentration projected to occur in association with a change in Pb intake or uptake. The slope parameter is factored by exposure parameters (e.g., exposure concentrations, environmental media intake rates) that relate exposure to blood Pb concentration ([Maddaloni et al., 2005](#); [U.S. EPA, 2003c](#); [Abadin and Wheeler, 1997](#); [Stern, 1996](#); [Bowers et al., 1994](#); [Stern, 1994](#); [Carlisle and Wade, 1992](#)). In slope factor models, Pb biokinetics are represented as a linear function between the blood Pb concentration and either Pb uptake (uptake slope factor, USF) or Pb intake (intake slope factor, ISF). The models take the general mathematical forms:

$$\text{PbB} = \text{E} \times \text{ISF}$$

Equation 3-2

$$\text{PbB} = \text{E} \times \text{AF} \times \text{USF}$$

Equation 3-3

where PbB is the blood Pb concentration, E is an expression for exposure (e.g., soil intake $\times$ 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 rather than absorbed Pb and, therefore, integrate both absorption and biokinetics into a single slope factor, whereas models that utilize an uptake slope factor include a separate absorption parameter. In contrast to mechanistic models, slope factor models predict quasi-steady state blood Pb concentrations that correspond to time-averaged daily Pb intakes (or uptakes) that occur over sufficiently long periods to produce a quasi-steady state (i.e., >75 days, ~ 3 times the $t_{1/2}$ for elimination of Pb in blood).

The U.S. EPA *Adult Lead Methodology* (ALM) is an example of a slope factor model that has had extensive regulatory use in the EPA Superfund program for assessing health risks to adults associated with non-residential exposures to Pb in contaminated soils ([Maddaloni et al., 2005](#); [U.S. EPA, 1996a](#)). The model was developed to predict maternal and fetal blood Pb concentrations that might occur in relation to maternal exposures to contaminated soils. The model assumes an uptake slope factor of 0.4 µg/dL blood per µg/day Pb uptake. Additional discussion of slope factor models that have been used or proposed for regulatory use can be found in the 2006 Pb AQCD ([U.S. EPA, 2006b](#)).

Previous studies included in the 2006 Pb AQCD ([U.S. EPA, 2006b](#)) explored the relationship between blood Pb in children and environmental Pb concentrations. In a pooled analysis of 12 epidemiologic studies, interior dust Pb loading, exterior soil/dust Pb, age, mouthing behavior, and race were all statistically significant variables included in the regression model for blood Pb concentration ([Lanphear et al., 1998](#)). Significant interactions were found for age and dust Pb loading, mouthing behavior and exterior soil/dust level, and SES and water Pb level. In a meta-analysis of 11 epidemiologic studies, among children the most common exposure pathway influencing blood Pb concentration in structural equation modeling was exterior soil, operating through its effect on interior dust Pb and hand Pb ([Succop et al., 1998](#)). Similar to Lanphear et al. (1998), in the linear regression model, interior dust Pb loading had the strongest relationships with blood Pb concentration. Individual studies conducted in Rochester, NY, Cincinnati, OH, and Baltimore, MD report similar relationships between children's blood Pb and interior dust concentrations ([Lanphear and Roghmann, 1997](#); [U.S. EPA, 1996b](#); [Bornschein et al., 1985](#)).

Dixon et al. (2009) reported a multivariate analysis of associations between environmental Pb concentrations and blood Pb concentrations, based on data collected in the NHANES (1999-2004). The analyses included 2,155 children, age 12-60 months. The population-weighted geometric mean blood Pb concentration was 2.03 µg/dL (GSD 1.03). A linear model applied to these data yielded an R^2 of 40% ([Table 3-17](#)). The regression coefficient for the relationship between $\ln[\text{blood Pb concentration (}\mu\text{g/dL)}]$ and $\ln[\text{floor dust Pb concentration (}\mu\text{g/ft}^2\text{)}]$ was 0.386 (SE 0.089) for “not smooth and cleanable” surfaces (e.g., high-pile carpets) and 0.205 (SE 0.032) for “smooth and cleanable” surfaces (e.g., uncarpeted or low-pile carpets). These coefficients correspond to a 2.4-fold or 1.6-fold increase in blood Pb concentration, respectively, for a 10-fold increase in floor dust Pb concentration.

Table 3-17 Linear model relating environmental Pb exposure and blood Pb concentration in children.

Variables	Overall p-value	Levels ^a	Estimate (SE)	p-Value
Intercept	0.172		- 0.517 (0.373)	0.172
Age (in years)	<0.001	Age	2.620 (0.628)	<0.001
		Age ²	-1.353 (0.354)	<0.001
		Age ³	0.273 (0.083)	0.002
		Age ⁴	-0.019 (0.007)	0.008
Year of construction	0.014	Intercept for missing	-0.121 (0.052)	0.024
		1990–present	-0.198 (0.058)	0.001
		1978–1989	-0.196 (0.060)	0.002
		1960–1977	-0.174 (0.056)	0.003
		1950–1959	-0.207 (0.065)	0.003
		1940–1949	-0.012 (0.072)	0.870
		Before 1940	0.000	—
PIR	<0.001	Intercept for missing	0.053 (0.065)	0.420
		Slope	-0.053 (0.012)	<0.001
Race/ethnicity	<0.001	Non-Hispanic white	0.000	—
		Non-Hispanic black	0.247 (0.035)	<0.001
		Hispanic	-0.035 (0.030)	0.251
		Other	0.128 (0.070)	0.073
Country of birth	0.002	Missing	-0.077 (0.219)	0.728
		U.S. ^b	0.000	—
		Mexico	0.353 (0.097)	<0.001
		Elsewhere	0.154 (0.121)	0.209
Floor surface/condition × log floor PbD	<0.001	Intercept for missing	0.178 (0.094)	0.065
		Not smooth and cleanable	0.386 (0.089)	<0.001
		Smooth and cleanable or carpeted	0.205 (0.032)	<0.001
Floor surface/condition × (log floor PbD) ²		Not smooth and cleanable	0.023 (0.015)	0.124
		Smooth and cleanable or carpeted	0.027 (0.008)	0.001
Floor surface/condition × (log floor PbD) ³		Uncarpeted not smooth and cleanable	-0.020 (0.014)	0.159
		Smooth and cleanable or carpeted	-0.009 (0.004)	0.012
Log windowsill PbD	0.002	Intercept for missing	0.053 (0.040)	0.186
		Slope	0.041 (0.011)	<0.001

Table 3-17 (Continued): Linear model relating environmental Pb exposure and blood Pb concentration in children.

Variables	Overall p-value	Levels ^a	Estimate (SE)	p-Value
Home-apartment type	<0.001	Intercept for missing	−0.064 (0.097)	0.511
		Mobile home or trailer	0.127 (0.067)	0.066
		One family house, detached	−0.025 (0.046)	0.596
		One family house, attached	0.069 (0.060)	0.256
		Apartment (1–9 units)	−0.133 (0.056)	0.022
		Apartment (≥ 10 units)		
Anyone smoke inside the home	0.015	Missing	0.138 (0.140)	0.331
		Yes	0.100 (0.040)	0.015
		No	0.000	-
Log cotinine concentration (ng/dL)	0.004	Intercept for missing	−0.150 (0.063)	0.023
		Slope	0.039 (0.012)	0.002
Window, cabinet, or wall renovation in a pre-1978 home	0.045	Missing	−0.008 (0.061)	0.896
		Yes	0.097 (0.047)	0.045
		No	0.000	—

^aChildren N = 2,155 (age 10-60 months); R² = 40%

^bIncludes the 50 states and the District of Columbia

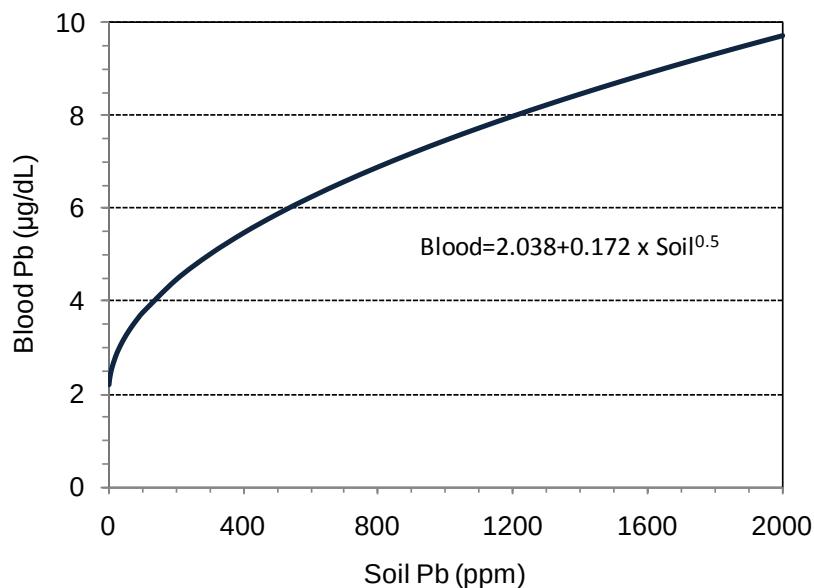
Source: Dixon et al. (2009).

Mielke et al. (2007a) analyzed blood Pb and soil Pb concentration data collected as part of a blood Pb screening program in New Orleans, Louisiana (2000-2005). The data set included 55,551 blood Pb measurements for children 0-6 years of age and 5,467 soil Pb measurements. Blood Pb and soil Pb concentrations were matched at the level of census tracts. The association between blood Pb concentration and soil Pb concentration was evaluated using nonparametric permutation methods. The resulting best-fit model was:

$$\text{PbB} = 2.038 + (0.172 \times \text{PbS}^{0.5})$$

Equation 3-4

where PbB is the median blood Pb concentration and PbS is the median soil Pb concentration. Although the overall association between blood Pb and soil Pb was strong (R²=0.528), there was considerable scatter in the data. For example, at the median soil Pb levels of 100 and 500 mg/kg, median blood Pb ranged from 2 to 8 µg/dL and 3 to 12 µg/dL, respectively. The resulting curvilinear relationship predicts a twofold increase in blood Pb concentration for an increase in soil Pb concentration from 100 to 1,000 ppm (Figure 3-26).



Note: The data set included 55,551 blood Pb measurements for children 0-6 years of age and 5,467 soil Pb measurements. Blood Pb and soil Pb concentrations were matched at the level of census tracts ([Mielke et al., 2007a](#)).

Figure 3-26 Predicted relationship between soil Pb concentration and blood Pb concentration in children based on data collected in New Orleans, Louisiana: 2000-2005.

In a subsequent re-analysis of the New Orleans (2000-2005) data, individual child blood Pb observations were matched to census tract soil concentrations ([Zahran et al., 2011](#)). This analysis confirmed the association between blood Pb and both soil Pb and age reported in Mielke et al. ([2007a](#)). Regression coefficients for soil Pb (random effects generalized least squares regression) ranged from 0.217 to 0.214 (per soil Pb^{0.5}), which is equivalent to approximately a 2-fold increase in blood Pb concentration for an increase in soil Pb concentration from 100 to 1,000 ppm.

Several studies have linked elevated blood Pb levels to residential soil exposures for populations living nearby industrial or mining facilities. Gulson et al. ([2009](#)) studied the blood Pb and isotopic Pb ratios of children younger than 5-years old and adults older than 18-years old living in the vicinity of a mine producing Magellan Pb ore in western Australia. They observed a median blood Pb level of 6.6 µg/dL for the children, with isotopic ratios indicating contributions from the mine ranging from 27 to 93%. A weak but significant linear association between blood Pb level and percent Magellan Pb was observed ($R^2 = 0.12$, $p = 0.018$). Among children with blood Pb levels over 9 µg/dL and among adults, the isotopic ratios revealed Pb exposures from a variety of sources.

Garavan et al. (2008) measured soil Pb and blood Pb levels among children aged 1-month to 17.7-years old in an Irish town near a coal mine. The blood Pb measurements were instituted as part of a screening and community education program given that the presence of Pb had been documented in the environment. Garavan et al. (2008) found that over 3 years of the screening period, median blood Pb levels reduced by roughly 22% from 2.7 to 2.1 µg/dL.

An extensive discussion of the relationships between environmental Pb levels and blood Pb concentrations in children at the Bunker Hill Superfund Site location, a former Pb mining and smelting site, was provided in the 2006 Pb AQCD (U.S. EPA, 2006c). In the most recent analysis (TerraGraphics Environmental Engineering, 2004) of the data on environmental Pb levels and child blood Pb concentrations (1988-2002), blood Pb concentrations (annual GM) ranged from 2.6 to 9.9 µg/dL. Environmental Pb levels (e.g., dust, soil, paint Pb levels) data were collected at ~3,000 residences, with interior dust Pb concentrations (annual GM) ranging from ~400 to 4,200 mg/kg and yard soil Pb concentration (annual GM) ranging from ~150 to 2,300 mg/kg. Several multivariate regression models relating environmental Pb levels and blood Pb concentration were explored; the model having the highest R² (0.26) is shown in Table 3-18. The model predicts significant associations between blood Pb concentration, age, interior dust, yard soil, neighborhood soil (geometric mean soil Pb concentration for areas within 200 ft of the residence), and community soil Pb concentration (community GM). Based on the standardized regression coefficients, the community soil Pb concentration had the largest effect on blood Pb concentration, followed by neighborhood soil Pb concentration, interior dust Pb concentration, and yard soil Pb concentration (Table 3-18). The model predicted a 1.8 µg/dL decrease in blood Pb concentration in association with a decrease in community soil Pb concentration from 2,000 to 1,000 mg/kg. The same decrease in neighborhood soil Pb concentration, interior dust Pb concentration, or yard soil Pb concentration was predicted to result in a 0.8, 0.5, or 0.2 µg/dL decrease in blood Pb concentration, respectively. Note that the soil Pb component of the model was similar to that derived by Lewin et al. (1999), in which a model of blood Pb as a function of soil Pb among 0-6 year old children living near one of four industrial sites was given as $PbB = 0.2438\ln(PbS) + 0.2758$.

Table 3-18 General linear model relating blood Pb concentration in children and environmental Pb levels—Bunker Hill Superfund Site.

Parameter	Coefficient	P-value	Standardized Coefficient
Intercept	-0.1801	0.7916	0.00000
Age (yr)	-0.4075	<0.0001	-0.2497
ln(interior dust Pb); (mg/kg)	0.7288	<0.0001	0.1515
ln(yard soil Pb); (mg/kg)	0.2555	0.0002	0.0777
GM soil Pb within 200 ft of residence (mg/kg)	0.0008	<0.0001	0.1380
GM community soil Pb (mg/kg)	0.0018	<0.0001	0.2250

$R^2 = 0.264$; $p < 0.0001$; based on data from Bunker Hill Superfund Site, collected over the period 1988-2002.

GM: geometric mean; ln: natural log.

Source: TerraGraphics (2004).

Malcoe et al. (2002) analyzed 1997 data on blood Pb and environmental Pb concentrations in a representative sample of Native American and white children (N = 224, age 1-6 years) who resided in a former Pb mining region in Ottawa County, OK. The data set included measurements of blood Pb, yard soil Pb, residential interior dust Pb loading, first-draw water Pb, paint Pb assessment and other behavioral (i.e., hand-to-mouth activity, hygiene rating) and demographic variables (i.e., hygiene rating, poverty level, caregiver education). A multivariate regression model accounted for 34% of the observed variability in blood Pb. Yard soil Pb and interior dust Pb loading accounted for 10% and 3% of the blood Pb variability, respectively. The regression model predicted a slope of 0.74 $\mu\text{g/dL}$ blood Pb per $\ln[\mu\text{g/g soil Pb}]$ and a slope of 0.45 $\mu\text{g/dL}$ blood Pb per $\ln[\mu\text{g/ft}^2]$ dust Pb loading.

3.6 Biokinetic Models of Pb Exposure-Blood Pb Relationships

An alternative to regression models are mechanistic models, which attempt to specify all parameters needed to describe the mechanisms (or processes) of transfer of Pb from the environment to human tissues. Such mechanistic models are more complex than regression models; this added complexity introduces challenges in terms of their mathematical solution and empirical verification. However, by incorporating parameters that can be expected to vary spatially or temporally, or across individuals or populations, mechanistic models can be extrapolated to a wide range of exposure scenarios, including those that may be outside of the domain of paired predictor-outcome data used to develop the model. Exposure-intake models, a type of mechanistic models, are highly simplified

mathematical representations of relationships between levels of Pb in environmental media and human Pb intakes (e.g., μg Pb ingested per day). These models include parameters representing processes of Pb transfer between environmental media (e.g., air to surface dust) and to humans, including rates of human contact with the media and intakes of the media (e.g., g soil ingested per day). Intake-biokinetic models provide the analogous mathematical representation of relationships between Pb intakes and Pb levels in body tissues (e.g., blood Pb concentration). Biokinetic models include parameters that represent processes of Pb transfer (a) from portals of entry into the body and (b) from blood to tissues and excreta. Linked together, exposure-intake and intake-biokinetics models (i.e., integrated exposure-intake-biokinetics models) provide an approach for predicting blood Pb concentrations (or Pb concentrations in other tissues) that corresponds to a specified exposure (medium, concentration, and duration). Detailed information on exposure and internal dose can be obtained from controlled experiments, but almost never from epidemiological observations or from public health monitoring programs. Exposure intake-biokinetics models can provide these predictions in the absence of complete information on the exposure history and blood Pb concentrations for an individual (or population) of interest. Therefore, these models are critical to applying epidemiologic-based information on blood Pb-response relationships to the quantification and characterization of human health risk. These models are also critical for assessing the potential impacts of public health programs directed at mitigation of Pb exposure or of remediation of contaminated sites.

However, these models are not without their limitations. Human exposure-biokinetics models include large numbers of parameters, which are required to describe the many processes that contribute to Pb intake, absorption, distribution, and elimination. The large number of parameters complicates the assessment of confidence in individual parameter values, many of which cannot be directly measured. Statistical procedures can be used to evaluate the degree to which model outputs conform to “real-world” observations and values of influential parameters can be statistically estimated to achieve good agreement with observations. Still, uncertainty may be expected to remain about parameters in complex exposure-biokinetic models. Such uncertainties need to be identified and their impacts on model predictions quantified (i.e., sensitivity analysis or probabilistic methods).

Modeling of human Pb exposures and biokinetics has advanced considerably during the past several decades, although there have been relatively few developments since the 2006 Pb AQCD was published. Still in use are the *Integrated Exposure Uptake Biokinetic (IEUBK) Model for Lead in Children* ([U.S. EPA, 1994](#)) and models that simulate Pb biokinetics in humans from birth through adulthood ([O'Flaherty, 1995](#); [Leggett, 1993](#);

[O'Flaherty, 1993](#)). The EPA AALM is still in development. A complete and extensive discussion of these models can be found in the 2006 Pb AQCD ([U.S. EPA, 2006b](#)).

3.7 Summary and Conclusions

3.7.1 Exposure

Exposure data considered in this assessment build upon the conclusions of the 2006 Pb AQCD ([2006b](#)), which found that air Pb concentrations in the U.S. and associated biomarkers of exposure to Pb have decreased substantially following reductions in industrial point sources of Pb, and restrictions on Pb in gasoline, house-hold paints, and solder. Pb exposure is difficult to assess because Pb has multiple sources in the environment and passes through various media. The atmosphere is the main environmental transport pathway for Pb, and, on a global scale, atmospheric Pb is primarily associated with fine particulate matter, which can deposit to soil and water. In addition to primary emission of particle-bearing or gaseous Pb to the atmosphere, Pb can be suspended to the air from soil or dust. Air-related pathways of Pb exposure are the focus of this assessment. In addition to inhalation of Pb from ambient air, air-related Pb exposure pathways include inhalation and ingestion of Pb from indoor dust and/or outdoor soil that originated from recent or historic ambient air (e.g., air Pb that has penetrated into the residence either via the air or tracking of soil), ingestion of Pb in drinking water contaminated from atmospheric deposition onto surface waters or from indirect surface runoff of deposition of ambient air Pb, and ingestion of Pb in dietary sources after uptake by plants or grazing animals. Non-air-related Pb exposures may include occupational exposures, hand-to-mouth contact with Pb-containing consumer goods, hand-to-mouth contact with dust or chips of peeling Pb-containing paint, or ingestion of Pb in drinking water conveyed through Pb pipes. Pb can cycle through multiple media prior to human exposure. Given the multitude of possible air-related exposure scenarios and the related difficulty of constructing Pb exposure histories, most studies of Pb exposure through air, water, and soil can be informative to this review. Other exposures, such as occupational exposures, contact with consumer goods in which Pb has been used, or ingestion of Pb in drinking water conveyed through Pb pipes may also contribute to Pb body burden.

A number of monitoring and modeling techniques have been employed for environmental Pb exposure assessment. Environmental Pb concentration data can be collected from ambient air Pb monitors, soil Pb samples, dust Pb samples, and dietary Pb samples to estimate human exposure. Exposure estimation error depends in part on the collection

efficiency of these methods; collection efficiency for ambient air Pb FRM samplers is described in [Section 2.4](#). Models, such as the Integrated Exposure Uptake Biokinetic (IEUBK) model, simulate human exposure to Pb from multiple sources and through various routes including inhalation, ingestion, and dermal exposure. IEUBK model inputs include soil Pb concentration, air Pb concentration, dietary Pb intake including drinking water, Pb dust ingestion, human activity, and biokinetic factors. Measurements and/or assumptions can be utilized when formulating the model inputs; errors in measurements and assumptions thus have the potential to propagate through the exposure models.

[Section 3.1](#) presents data illustrating potential exposure pathways. Soil can act as a reservoir for deposited Pb emissions, and exposure to soil contaminated with deposited Pb can occur through resuspended PM as well as hand-to-mouth contact, which is the main pathway of childhood exposure to Pb. Airborne particles containing Pb tend to be small (much of the distribution $<10\ \mu\text{m}$) compared with Pb in soil or dust particles ($\sim 50\ \mu\text{m}$ to several hundred μm); Pb deposition to soil is described in [Section 2.3](#). Hence, hand-to-mouth contact with Pb-bearing soil or dust and/or tracking Pb contaminated soil or dust into homes are more common means for human exposure to Pb. Infiltration of Pb dust into indoor environments has been observed, and Pb dust has been shown to persist in indoor environments even after repeated cleanings. Measurements of particle-bound inhalation Pb exposures reported in this assessment have shown that personal inhalation exposure measurements for air Pb concentration are typically higher than indoor or outdoor ambient air Pb concentrations.

3.7.2 Toxicokinetics

The majority of Pb in the body is found in bone (roughly 90% in adults, 70% in children); only about 1% of Pb is found in the blood. Pb in blood is primarily ($\sim 99\%$) bound to red blood cells (RBCs). It has been suggested that the small fraction of Pb in plasma ($<1\%$) may be the more biologically labile and toxicologically active fraction of the circulating Pb. The relationship between Pb in blood and plasma is pseudo-linear at relatively low daily Pb intakes (i.e., $<10\ \mu\text{g}/\text{kg}$ per day) and at blood Pb concentrations $<25\ \mu\text{g}/\text{dL}$, and becomes curvilinear at higher blood Pb concentrations due to saturable binding to RBC proteins. As blood Pb level increases and the higher affinity binding sites for Pb in RBCs become saturated, a larger fraction of the blood Pb is available in plasma to distribute to brain and other Pb-responsive tissues.

The burden of Pb in the body may be viewed as divided between a dominant slow (i.e., uptake and elimination) compartment (bone) and smaller fast compartment(s) (soft tissues). Pb uptake and elimination in soft tissues is much faster than in bone. Pb

accumulates in bone regions undergoing the most active calcification at the time of exposure. During infancy and childhood, bone calcification is most active in trabecular bone (e.g., patella); whereas, in adulthood, calcification occurs at sites of remodeling in cortical (e.g., tibia) and trabecular bone ([Aufderheide and Wittmers, 1992](#)). A high bone formation rate in early childhood results in the rapid uptake of circulating Pb into mineralizing bone; however, in early childhood bone Pb is also recycled to other tissue compartments or excreted in accordance with a high bone resorption rate ([O'Flaherty, 1995](#)). Thus, much of the Pb acquired early in life is not permanently fixed in the bone.

The exchange of Pb from plasma to the bone surface is a relatively rapid process. Pb in bone becomes distributed in trabecular and the more dense cortical bone. The proportion of cortical to trabecular bone in the human body varies by age, but on average is about 80% cortical to 20% trabecular. Of the bone types, trabecular bone is more reflective of recent exposures than is cortical bone due to the slow turnover rate and lower blood perfusion of cortical bone. Some Pb diffuses to kinetically deeper bone regions where it is relatively inert, particularly in adults. These bone compartments are much more labile in infants and children than in adults as reflected by half-times for movement of Pb from bone into the plasma (e.g., cortical half-time = 0.23 years at birth, 3.7 years at 15 years of age, and 23 years in adults; trabecular half-time = 0.23 years at birth, 2.0 years at 15 years of age, and 3.8 years in adults) ([Leggett, 1993](#)).

Evidence for maternal-to-fetal transfer of Pb in humans is derived from cord blood to maternal blood Pb ratios. Group mean ratios range from about 0.7 to 1.0 at the time of delivery for mean maternal blood Pb levels ranging from 1.7 to 8.6 µg/dL. Transplacental transfer of Pb may be facilitated by an increase in the plasma/blood Pb concentration ratio during pregnancy. Maternal-to-fetal transfer of Pb appears to be related partly to the mobilization of Pb from the maternal skeleton.

The dominant elimination phase of Pb kinetics in the blood, exhibited shortly after a change in exposure occurs, has a half-life of ~20-30 days. An abrupt change in Pb uptake gives rise to a relatively rapid change in blood Pb, to a new quasi-steady state, achieved in ~75-100 days (i.e., 3-4 times the blood elimination half-life). A slower phase of Pb clearance from the blood may become evident with longer observation periods following a decrease in exposure due to the gradual redistribution of Pb among bone and other compartments.

3.7.3 Pb Biomarkers

Overall, trends in blood Pb levels have been decreasing among U.S. children and adults over the past 35 years ([Section 3.4](#)). The median blood Pb level for the entire U.S.

population is 1.1 µg/dL and the 95th percentile blood Pb level was 3.3 µg/dL, based on the 2009-2010 NHANES data ([CDC, 2013](#)). Among children aged 1-5 years, the median and 95th percentiles were slightly higher at 1.2 µg/dL and 3.4 µg/dL, respectively.

Blood Pb is dependent on both the recent exposure history of the individual, as well as the long-term exposure history that determines body burden and Pb in bone. The contribution of bone Pb to blood Pb changes depending on the duration and intensity of the exposure, age, and various other physiological stressors that may affect bone remodeling (e.g., nutritional status, pregnancy, menopause, extended bed rest, hyperparathyroidism) beyond that which normally and continuously occurs. In children, largely due to faster exchange of Pb to and from bone, blood Pb is both an index of recent exposure and potentially an index of body burden. In adults and children, where exposure to Pb has effectively ceased or greatly decreased, a slow decline in blood Pb concentrations over the period of years is most likely due to the gradual release of Pb from bone. Bone Pb is an index of cumulative exposure and body burden. Even bone compartments should be recognized as reflective of differing exposure periods with Pb in trabecular bone exchanging more rapidly than Pb in cortical bone with the blood. This difference in the compartments makes Pb in cortical bone a better marker of cumulative exposure and Pb in trabecular bone more likely to be correlated with blood Pb, even in adults.

Sampling frequency is an important consideration when evaluating blood Pb and bone Pb levels in epidemiologic studies, particularly when the exposure is not well characterized. It is difficult to determine what blood Pb is reflecting in cross-sectional studies that sample blood Pb once, whether recent exposure or movement of Pb from bone into blood from historical exposures. In contrast, cross-sectional studies of bone Pb and longitudinal samples of blood Pb concentrations over time provide more of an index of cumulative exposure and are more reflective of average Pb body burdens over time. The degree to which repeated sampling will reflect the actual long-term time-weighted average blood Pb concentration depends on the sampling frequency in relation to variability in exposure. High variability in Pb exposures can produce episodic (or periodic) oscillations in blood Pb concentration that may not be captured with low sampling frequencies. Furthermore, similar blood Pb concentrations in two individuals (or populations), regardless of their age, do not necessarily translate to similar body burdens or similar exposure histories.

The concentration of Pb in urine follows blood Pb concentration, in that it mainly reflects the exposure history of the previous few months and therefore, is likely a relatively poor index of Pb body burden. There is added complexity with Pb in urine because concentration is also dependent upon urine flow rate, which requires timed urine samples

that is often not feasible in epidemiologic studies. Other biomarkers have been utilized to a lesser extent (e.g., Pb in teeth).

3.7.4 Air Lead-Blood Lead Relationships

The 1986 Pb AQCD ([U.S. EPA, 1986a](#)) described epidemiological studies of relationships between air Pb and blood Pb. Much of the pertinent earlier literature for child populations described in the 1986 Pb AQCD was also included in a meta-analysis by Brunekreef ([1984](#)). Based on the studies available at that time, the 1986 Pb AQCD concluded that “the blood Pb versus air Pb slope β is much smaller at high blood and air levels.” This is to say that the slope β was much smaller for occupational exposures where high blood Pb levels ($>40 \mu\text{g/dL}$) and high air Pb levels (much greater than $10 \mu\text{g/m}^3$) prevailed relative to lower environmental exposures which showed lower blood Pb and air Pb concentrations ($<30 \mu\text{g/dL}$ and $<3 \mu\text{g/m}^3$). For those environmental exposures, it was concluded that the relationship between blood Pb and air Pb “...for direct inhalation appears to be approximately linear in the range of normal ambient exposures ($0.1\text{--}2.0 \mu\text{g/m}^3$)” (Chapter 1, pp 98 of the 1986 Pb AQCD). In addition to the meta-analysis of Brunekreef ([1984](#)), more recent studies have provided data from which estimates of the blood Pb-air Pb slope can be derived for children ([Table 3-12](#)). The range of estimates from these studies is $4\text{--}9 \mu\text{g/dL}$ per $\mu\text{g/m}^3$, which encompasses the estimate from the Brunekreef ([1984](#)) meta-analysis. Most studies have described the blood Pb-air Pb relationship as either log-log ([Schnaas et al., 2004](#); [Hayes et al., 1994](#); [Brunekreef, 1984](#)), which predicts an increase in the blood Pb-air Pb slope with decreasing air Pb concentration or linear ([Hilts, 2003](#); [Tripathi et al., 2001](#); [Schwartz and Pitcher, 1989](#)), which predicts a constant blood Pb-air Pb slope regardless of air Pb concentrations. These differences may simply reflect model selection by the investigators; alternative models are not reported in these studies. The blood Pb-air Pb slope may also be affected in some studies by the inclusion of parameters (e.g., soil Pb) that may account for some of the variance in blood Pb attributable to air Pb. Other factors that likely contribute to the derived blood Pb-air Pb slope include differences in the populations examined and Pb sources, which varied among individual studies.

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CHAPTER 4 INTEGRATED HEALTH EFFECTS OF LEAD EXPOSURE

4.1 Introduction

This chapter summarizes, integrates, and evaluates the evidence for the broad spectrum of health effects associated with exposure to Pb. The chapter begins ([Section 4.2](#)) with a discussion of the evidence for the modes of action that mediate the health effects of Pb, including those modes of action that are shared by all of the health effects evaluated in this ISA and those modes of action that are specific to particular endpoints. Subsequent sections comprise evaluations of the epidemiologic and toxicological evidence for the effects of Pb exposure on health outcomes related to nervous system effects ([Section 4.3](#)), cardiovascular effects ([Section 4.4](#)), renal effects ([Section 4.5](#)), immune effects ([Section 4.6](#)), hematological effects ([Section 4.7](#)), and reproductive and developmental effects ([Section 4.8](#)). [Section 4.9](#) reviews the evidence for the effects of Pb on other noncancer health outcomes, for which the cumulative bodies of evidence are smaller, including those related to the hepatic system ([Section 4.9.1](#)), gastrointestinal system ([Section 4.9.2](#)), endocrine system ([Section 4.9.3](#)), bone and teeth ([Section 4.9.4](#)), ocular health ([Section 4.9.5](#)), and respiratory system ([Section 4.9.6](#)). Chapter 4 concludes with a discussion of the evidence for the cancer effects of Pb ([Section 4.10](#)).

Individual sections for major outcome categories (e.g., nervous system, cardiovascular, renal) begin with a summary of conclusions from the 2006 Pb AQCD ([U.S. EPA, 2006c](#)) followed by an evaluation of recent (i.e., published since the completion of the 2006 Pb AQCD) studies that is intended to build upon evidence from previous reviews. Within each of these sections, results are organized into smaller groups of related endpoints (e.g., cognitive function, externalizing behaviors, neurodegenerative diseases) then specific scientific discipline (i.e., epidemiology, toxicology). This chapter evaluates evidence for both short- and long-term Pb exposures, which are defined as up to four weeks and greater than four weeks, respectively, in animal toxicological studies and up to one year and greater than one year, respectively, in epidemiologic studies ([Section 1.1](#)).

Sections for each of the major outcome categories (e.g., nervous system, cardiovascular, renal effects) conclude with an integrated summary of the assessment of evidence and conclusions regarding causality. A determination of causality was made for a group of related endpoints within a major outcome category (e.g., cognitive function, hypertension) by evaluating the evidence for each endpoint group independently with the causal framework (described in the [Preamble](#) to this ISA).

Judgments regarding causality were made by evaluating evidence for consistency of findings across multiple studies and the extent to which chance, confounding (i.e., bias due to a correlation with Pb biomarker level and causal association with the outcome), and other biases could be ruled out with reasonable confidence. Such evidence included high quality epidemiologic studies with representative population-based groups, prospective versus cross-sectional or ecologic design; rigorous statistical analysis (i.e., multivariate regression) with assessment of potential confounding factors; information on the concentration-response relationship; and supporting toxicological evidence. Epidemiologic studies varied in the extent to which they considered potential confounding. Because no single study considered all potential confounding factors, and not all potential confounding factors were examined in the collective body of evidence, residual confounding by unmeasured factors is possible. Residual confounding also is possible by factors measured with error. However, the evidence was evaluated for the extent to which there was examination of factors well documented in the literature to be associated with Pb exposure and health outcomes and supporting toxicological evidence to help minimize the undue influence of confounding bias. The biological plausibility provided by the coherence of evidence between toxicology and epidemiology and across a spectrum of related endpoints, including evidence for modes of action, was used as support to address uncertainties in the epidemiologic evidence due to biases from factors such as selective publication, recruitment or participation of subjects; reverse causality; or confounding.

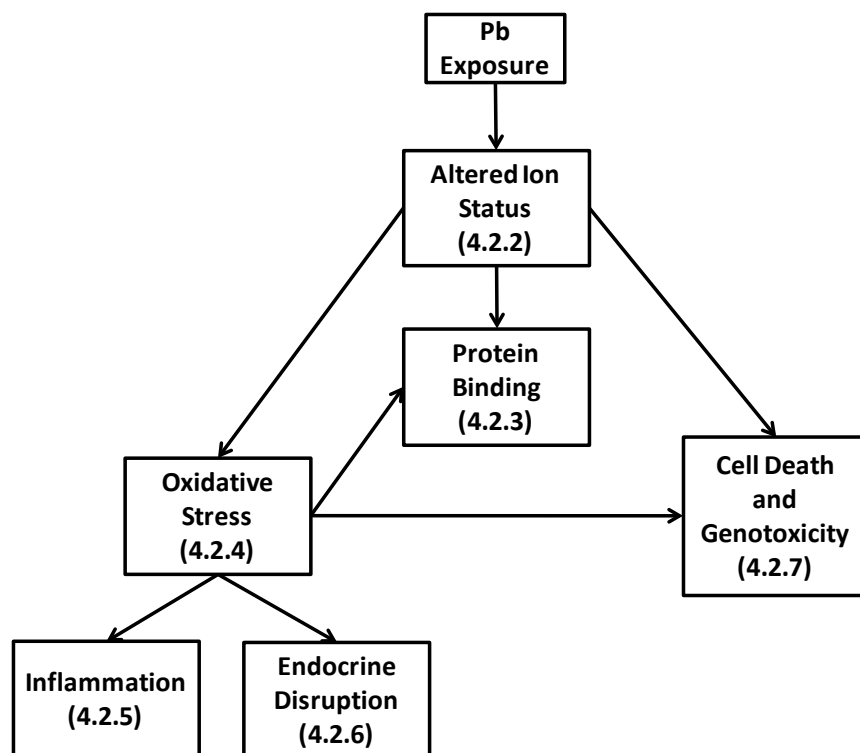
Judgments regarding causality also were made by evaluating the evidence for Pb exposure routes and the full range of concentrations in animal toxicological and epidemiologic studies considered relevant to this ISA, i.e., blood Pb levels up to 30 µg/dL for most outcomes as described in [Section 1.1](#). Studies that examined higher Pb concentrations were evaluated particularly to inform mode of action. For epidemiologic studies of adults that examined concurrent blood Pb levels, it is recognized that the associations observed with health effects likely are influenced by higher past blood Pb levels and Pb exposures ([Section 3.4.1](#)). Some data are available to inform past air Pb concentrations and blood Pb levels in the U.S. As discussed in [Section 2.2](#), peak U.S. use of Pb anti-knock additives in automobile gasoline and peak industrial emissions occurred between 1968 and 1972, and Pb in gasoline was finally banned from use in 1996. In addition to these changes, the annual average of the maximum 3-month average air Pb concentration for trends monitors across the U.S. decreased from 1.3 µg/m³ in 1980 to 0.14 µg/m³ in 2010 ([Section 2.5](#)). The mean 2010 3-month rolling average for non-source monitors (i.e., monitors not in close proximity to specific sources) was an order of magnitude lower than the 2010 trends site average. Studies of blood Pb during the time period of peak air Pb concentrations in the U.S. (1968-1980) show higher U.S. blood Pb levels than those in 2009-2010 ([Section 3.4](#)). Among subjects ages 1-74 years examined

in U.S. NHANES II (1976-1980) by Pirkle et al. ([1994](#)), the geometric mean blood Pb level was 12.8 µg/dL. Studies that examined blood Pb levels in various age groups reported geometric means in adults (ages 20-79 years in 1968-1980) of 13.1-16.2 µg/dL ([Pirkle et al., 1994](#); [Tepper and Levin, 1975](#)) and in children (ages 1 month-6 years in 1970-1980) of 15.0-30.4 µg/dL ([Pirkle et al., 1994](#); [Billick et al., 1979](#); [Fine et al., 1972](#)). These data indicate that for U.S. adults examined in the studies reviewed in this ISA, past blood Pb levels likely were within the range considered relevant for this ISA.

4.2 Modes of Action

4.2.1 Introduction

The diverse health effects associated with Pb exposure are dependent on multiple factors, including the concentration and duration of exposure, the particular Pb compounds constituting the exposure, and which tissues are affected. Pb exposure is linked to downstream health effects by various modes of action. A mode of action (MOA) is the common set of biochemical, physiological, or behavioral responses (i.e., empirically observable precursor steps) that can cumulatively result in the formation of negative health outcomes. Although the effects of Pb exposure appear to be mediated through multiple modes of action, alteration of cellular ion status (including disruption of calcium homeostasis, altered ion transport mechanisms, and perturbed protein function through displacement of metal cofactors) seems to be the major unifying mode of action underlying all subsequent modes of action ([Figure 4-1](#)). This section draws information from all of the subsequent health effects sections in Chapter 4, and identifies the major modes of action operating at the molecular, cellular, and tissue/organ level. In turn, the individual health effect sections bridge these MOA effects to those observed on the organismal level. Each of the individual health effect sections includes a more detailed description of the mechanisms specific to the individual health effect. Accordingly, this section differs in structure and content from other health effects sections as it does not primarily focus on the literature published since the 2006 Pb AQCD, but rather incorporates recent information with earlier studies (which together represent the current state of the science) on the possible modes of action of Pb. Higher concentrations of Pb are often utilized in mode of action studies. This section includes some studies that are conducted at concentrations greater than one order of magnitude above the upper end of the blood Pb distribution of the general U.S. population when it is likely that the mode of action does not differ at higher concentrations.



Note: The subsections where these MOAs are discussed are indicated in parentheses. ([Section 4.2.2](#); [Section 4.2.3](#); [Section 4.2.4](#); [Section 4.2.5](#); [Section 4.2.6](#); and [Section 4.2.7](#)).

Figure 4-1 Schematic representation of the relationships between the various MOAs by which Pb exposure exerts its health effects.

4.2.2 Altered Ion Status

Physiologically-relevant metal ions (e.g., Ca^{2+} , Mg, Zn, Fe) are known to have a multitude of functions in biological systems, including roles as charge carriers, intermediates in enzymatically-catalyzed reactions, and structural elements in the proper maintenance of tertiary protein conformations ([Garza et al., 2006](#)). It is through disruption of these biological functions that Pb exerts its negative actions, ultimately interfering with such tightly regulated processes as cell signaling, intracellular ion homeostasis, ion transport, energy metabolism, and enzymatic function.

4.2.2.1 Disruption of Ca^{2+} Homeostasis

Calcium (Ca^{2+}) is one of the most important carriers of cell signals and regulates virtually all aspects of cell function, including energy metabolism, signal transduction, hormonal regulation, cellular motility, and apoptosis ([Carafoli, 2005](#)). Ca^{2+} homeostasis is

maintained through a tightly regulated balance of cellular transport and intracellular storage ([Pentyala et al., 2010](#)). Disruption of Ca^{2+} homeostasis by Pb has been observed in a number of different cell types and cell-free environments, indicating that this is a major mode of action for Pb-induced toxicity on a cellular level.

Ca^{2+} homeostasis is particularly important in bone cells, as the skeletal system serves as the major dynamic reservoir of Ca^{2+} in the body ([Wiemann et al., 1999](#); [Long et al., 1992](#)). Bone cells also are unique in that they exist in a microenvironment that is high in Ca^{2+} , and potentially high in Pb concentrations. This may increase their relative exposure to Pb and thus Pb-induced effects ([Long et al., 1992](#)). A series of studies from the laboratory of Long, Dowd, and Rosen have indicated that exposure of cultured osteoblastic bone cells to Pb alters intracellular Ca^{2+} levels ($[\text{Ca}^{2+}]_i$). Exposure of osteoblasts to 1, 5, or 25 μM Pb for 40-300 minutes resulted in prolonged increases in $[\text{Ca}^{2+}]_i$ of 36, 50 and 120% over baseline, respectively ([Schanne et al., 1997](#); [Schanne et al., 1989](#)). Long et al. (1992) observed that exposure of osteoblasts to either 400 ng parathyroid hormone (PTH)/mL culture medium for 1 hour or 25 μM Pb for 20 hours increased $[\text{Ca}^{2+}]_i$. Pb-exposed cells pretreated with PTH increased $[\text{Ca}^{2+}]_i$ above concentrations observed in either single exposure (Pb alone or PTH alone), indicating that Pb may disrupt the ability of bone cells to respond to normal hormonal control. A similar increase in $[\text{Ca}^{2+}]_i$ was also observed when bone cells were co-treated with epidermal growth factor (EGF, 50 ng/mL) plus Pb (5 μM), versus EGF alone ([Long and Rosen, 1992](#)). Pb-induced increases in $[\text{Ca}^{2+}]_i$ were blocked by a protein kinase C (PKC) inhibitor, indicating that PKC activation may serve as one mechanism by which Pb perturbs $[\text{Ca}^{2+}]_i$ ([Schanne et al., 1997](#)). Schirmmacher et al. (1998) also observed alterations in Ca^{2+} homeostasis in osteoblasts exposed to 5 μM Pb for 50 minutes due to potential disruption of Ca^{2+} ATPases. However, Wiemann et al. (1999) demonstrated that exposure to 5 or 12.5 μM Pb inhibited the Ca^{2+} -release-activated calcium influx of Ca^{2+} independently of any inhibitory effect on Ca^{2+} ATPases.

Ca^{2+} homeostasis has also been shown to be disturbed in erythrocytes exposed to Pb ([Quintanar-Escorza et al., 2010](#); [Quintanar-Escorza et al., 2007](#); [Shin et al., 2007](#)). In blood samples taken from Pb-exposed workers (mean [SD] blood Pb level: 74.4 [21.9] $\mu\text{g/dL}$), the $[\text{Ca}^{2+}]_i$ was approximately 2.5-fold higher than that seen in nonexposed workers (mean [SD] blood Pb level: 9.9 [2.0] $\mu\text{g/dL}$) ([Quintanar-Escorza et al., 2007](#)). The increase in $[\text{Ca}^{2+}]_i$ was associated with higher osmotic fragility and modifications in erythrocyte shape. In a separate investigation, when erythrocytes from 10 healthy volunteers were exposed (in vitro) at concentrations of 0.2 to 6.0 μM Pb for 24 or 120 hours, concentration-related increases in $[\text{Ca}^{2+}]_i$ were observed across all concentrations for both durations of exposure ([Quintanar-Escorza et al., 2010](#)). Subsequent exposures of erythrocytes to either 0.4 or 4.0 μM Pb [corresponding to 10 or

80 µg/dL in exposed workers ([Quintanar-Escorza et al., 2007](#)) for 12-120 hours resulted in duration-related increases with durations >12 hours. Osmotic fragility (measured as percent hemolysis) was increased in erythrocytes exposed to 0.4 µM Pb for 24 hours. Co-incubation with a vitamin E analog mitigated these effects, indicating that the increase in $[Ca^{2+}]_i$ is dependent on the oxidative state of the erythrocytes. Shin et al. ([2007](#)) observed that incubation of human erythrocytes with 5 µM Pb for 1 hour resulted in a 30-fold increase in $[Ca^{2+}]_i$ in vitro, inducing the pro-coagulant activity of exposed erythrocytes. Induction of pro-coagulant activity in erythrocytes could lead to thrombus formation and negatively contribute to overall cardiovascular health; whereas increased osmotic fragility could substantially reduce erythrocyte life span and ultimately lead to anemic conditions.

Similar to effects seen in erythrocytes, Pb has been observed to interfere with Ca^{2+} homeostasis in platelets and white blood cells. Dowd and Gupta ([1991](#)) observed that 1 µM Pb (for 3.5 hours) was the lowest exposure concentration to result in increases in $[Ca^{2+}]_i$ in human platelets (in vitro). The observed increase in $[Ca^{2+}]_i$ levels was attributed to the increased influx of external Ca^{2+} , possibly through ligand-gated Ca^{2+} channels. In mouse splenic lymphocytes, 1 µM Pb was the lowest exposure concentration found to increase $[Ca^{2+}]_i$, with incubation periods of 10 minutes or greater ([Li et al., 2008c](#)). These increases in $[Ca^{2+}]_i$ appeared to be reversible as $[Ca^{2+}]_i$ returned to baseline after one hour. Pretreatment with a calmodulin antagonist slightly mitigated the effects of Pb exposure, indicating a role for calmodulin in disruption of Ca^{2+} homeostasis by Pb exposure in lymphocytes. In rat tail arteries exposed to 1.2 µM Pb acetate for 1 hour, $[Ca^{2+}]_i$ increased over controls, possibly through increased transmembrane influx of external Ca^{2+} ([Piccinini et al., 1977](#)).

Exposure of the microsomal fraction (prepared from rat brain cells) to as little as 0.25 µM Pb for 2 minutes resulted in increased release of Ca^{2+} into the culture medium ([Pentyala et al., 2010](#)). Further, Pb exposure also decreased the activity of microsomal Ca^{2+} ATPase, thus decreasing the sequestration of Ca^{2+} into microsomes. The results of this study suggest that Pb-induced disruption of microsomal release and re-uptake of Ca^{2+} may alter Ca^{2+} homeostasis, ultimately leading to altered signal transduction and neuronal dysfunction. However, Ferguson et al. ([2000](#)) observed that $[Ca^{2+}]_i$ was decreased in rat hippocampal neurons in response to exposure to 0.1 µM Pb for 1-48 hours; although the observed decreases were not time-dependent. The decrease in $[Ca^{2+}]_i$ was shown to be due to increased efflux of Ca^{2+} out of the neuron via a calmodulin-regulated mechanism, possibly through stimulated Ca^{2+} efflux via Ca^{2+} ATPase.

Pb exposure has been shown to disrupt $[Ca^{2+}]_i$ levels in multiple cell types including osteoblasts, erythrocytes, platelets, and neuronal cells. This alteration in Ca^{2+} homeostasis

could potentially affect cell signaling and disrupt the normal physiological function of these cells.

4.2.2.2 Disruption of Ion Transport Mechanisms

As described above, deregulation of Ca^{2+} homeostasis can result in negative effects in multiple organ systems. Under normal conditions in the life cycle of most cells, cytosolic concentrations of free Ca^{2+} fluctuate between approximately 100 to 200 nM and Ca^{2+} that has entered the cell must be removed in order to maintain normal homeostatic concentrations ([Carafoli, 2005](#)). An important component in the maintenance of Ca^{2+} homeostasis is transmembrane transport of Ca^{2+} ions via Ca^{2+} ATPase and voltage-gated Ca^{2+} channels ([Carafoli, 2005](#)). Pb has been shown to disrupt the normal movement of Ca^{2+} ions, as well as other physiologically important ions through interactions with these transport mechanisms.

Multiple studies have reported alterations in the activity of Na^+/K^+ ATPase, Ca^{2+} ATPase, and Mg^{2+} ATPases after Pb exposure in animal models. Decreases in the activity of all three ATPases were observed in the kidneys and livers of rats exposed to 750 ppm Pb in drinking water for 11 weeks (mean [SD] blood Pb level: 55.6 [6.3] $\mu\text{g/dL}$) ([Kharoubi et al., 2008a](#)) and in erythrocytes from rats exposed to 2,000 ppm Pb in drinking water for 5 weeks (mean [SD] blood Pb level: 97.56 [11.8] $\mu\text{g/dL}$) ([Sivaprasad et al., 2003](#)). Increases in lipid peroxidation were seen in both studies, and the decrements in ATPase activities may be explained by generation of free radicals in Pb-exposed animals. A decrease in the activity of Na^+/K^+ ATPase was observed in rabbit kidney membranes exposed to 0.01 to 10 μM Pb, possibly due to Pb inhibiting the hydrolytic cleavage of phosphorylated intermediates in the K-related branch of the pump ([Gramigni et al., 2009](#)). Similar decreases in Na^+/K^+ ATPase activity were observed in brain synaptosomes isolated from rats that were exposed to 200 ppm Pb in drinking water for 3 months (blood Pb level: 37.8 $\mu\text{g/dL}$) ([Rafalowska et al., 1996](#)) or 15 mg Pb/kg injected (i.p.) for 7 days (blood Pb level: 112.5 $\mu\text{g/dL}$) ([Struzynska et al., 1997a](#)). Inhibition of Na^+/K^+ ATPase activity was also observed in primary cerebellar granule neuronal cultures obtained from rat pups that were pre- and post-natally (to PND8) exposed to Pb (1,000 ppm Pb acetate in dams' drinking water, resulting in blood Pb level of 4 $\mu\text{g/dL}$) ([Baranowska-Bosiacka et al., 2011b](#)). The activity of Ca^{2+} ATPase in the sarcoplasmic reticulum of rabbits exposed to 0.01 μM Pb was similarly decreased ([Hechtenberg and Beyersmann, 1991](#)). The inhibitory effect of Pb was diminished in the presence of high Mg-ATP concentrations. The activity of generic ATPase was reported to be altered in the testes of rat pups exposed to 300 ppm (mg/L) Pb acetate, both during lactation and in drinking water after weaning to the age of 6, 8, 10, or 12 weeks ([Liu et al., 2008](#)). In pregnant rats

fed a Pb-depleted ($20 \pm 5 \mu\text{g/kg}$) or control (1 mg/kg) diet during gestation and lactation, no difference was observed in the activity of Na^+/K^+ ATPase and $\text{Ca}^{2+}/\text{Mg}^{2+}$ ATPase in the parental generation ([Eder et al., 1990](#)). However, the offspring (exposed via placental and lactational transfer of Pb) of Pb-depleted rats displayed decreased activities in both enzymes compared with offspring of rats with higher Pb exposures. An increase in the Na^+/K^+ ATPase activity was observed in rats treated (i.p.) with 20 mg/kg Pb for 14 consecutive days ([Jehan and Motlag, 1995](#)). Co-exposure of Pb with Zn and Cu greatly attenuated the increase in ATPase activity. Although the precise mechanism was not investigated, Navarro-Moreno et al. ([2009](#)) reported that Ca^{2+} uptake was diminished in proximal renal tubule cells in rats chronically exposed to 500 ppm Pb in drinking water for 7 months (mean [SD] blood Pb level: $43.0 [7.6] \mu\text{g/dL}$).

In vitro studies of ATPase activities in human erythrocyte ghosts have also shown that Pb affects the transport of metal ions across membranes. Calderon-Salinas et al. ([1999a](#)) observed that $1\text{-}5 \times 10^3 \mu\text{M}$ Pb and Ca^{2+} were capable of inhibiting the passive transport of each other in human erythrocyte ghosts incubated with both cations. Subsequent inhibition experiments indicated that both cations share the same electrogenic transport pathway ([Sakuma et al., 1984](#)). Further study by this group ([Calderón-Salinas et al., 1999b](#)) demonstrated that Pb can noncompetitively block the transport of Ca^{2+} by inhibiting the activity of $\text{Ca}^{2+}/\text{Mg}^{2+}$ ATPase at concentrations of $1\text{-}5 \times 10^3 \mu\text{M}$. Mas-Oliva ([1989](#)) demonstrated that the activity of $\text{Ca}^{2+}/\text{Mg}^{2+}$ ATPase in human erythrocyte ghosts was inhibited by incubation with $0.1\text{-}100 \mu\text{M}$ Pb. The inhibitory action was most likely due to direct reaction with sulfhydryl groups on the ATPase enzyme at Pb concentrations greater than $1 \mu\text{M}$, but due to the action of Pb on calmodulin at lower concentrations. Grabowska and Guminska ([1996](#)) observed that $10 \mu\text{g/dL}$ was the lowest Pb concentration to decrease the activity of Na^+/K^+ ATPase in erythrocyte ghosts; activity of $\text{Ca}^{2+}/\text{Mg}^{2+}$ ATPase was less sensitive to Pb exposure, and Mg^{2+} ATPase activity was not affected.

Effects on ATPase activity are also observed in association with blood Pb levels in human populations. In a study investigating ATPase activities in Pb-exposed workers in Nigeria, Abam et al. ([2008](#)) observed that the activity of erythrocyte membrane-bound $\text{Ca}^{2+}/\text{Mg}^{2+}$ ATPase was decreased by roughly 50% in all occupational groups (range of mean [SD] blood Pb level across nine occupational groups: $28.75 [11.31]$ to $42.07 [12.01] \mu\text{g/dL}$) compared to nonexposed controls (mean [SD] blood Pb level: $12.34 [2.44]$ in males and $16.85 [6.01] \mu\text{g/dL}$ in females). Higher membrane concentrations of Ca^{2+} and Mg^{2+} were also observed, indicating that Pb prevented the efflux of those cations from the cell, most likely by substituting for those metals in the active site of the ATPase. In a study of 247 mother-newborn pairs, Campagna et al. ([2000](#)) observed that newborn cord blood Pb (geometric mean [5th-95th percentile]: $4.8 [2.8\text{-}9.2] \mu\text{g/dL}$) was

negatively and significantly associated with maternal blood Ca^{2+} pump activities; however, newborn cord blood Pb was not significantly associated with newborn (cord) blood Ca^{2+} pump activities. Newborn hair Pb (geometric mean [5th-95th percentile]: 1.1 [0.1-8.0] $\mu\text{g/g}$) was negatively and significantly associated with both maternal and newborn cord blood Ca^{2+} pump activities. In a population of 81 newborns, Huel et al. (2008) found that newborn hair and newborn cord blood Pb levels (mean [SD] newborn hair Pb and newborn cord blood Pb levels: 1.22 [1.41] $\mu\text{g/g}$ and 3.54 [1.72] $\mu\text{g/dL}$) were negatively associated with Ca^{2+} ATPase activity in plasma membranes of erythrocytes isolated from newborn cord blood; newborn hair Pb levels were more strongly associated with newborn cord Ca^{2+} pump activity than were newborn cord blood Pb levels.

Pb has also been shown to disrupt cation transport mechanisms through direct action on voltage-gated cation channels. Audesirk and Audesirk (1993, 1991) demonstrated that extracellular free Pb inhibits the action of multiple voltage-gated Ca^{2+} channels, with free Pb IC_{50} (half maximal inhibitory concentration) values of 0.7 μM for L-type channels and 1.3 μM for T-type channels in neuroblastoma cells maintained in culture media, and IC_{50} values as low as 0.03 μM for L-type channels in cultured hippocampal neurons. Sun and Suszkiw (1995) corroborated the inhibitory action of extracellular Pb on voltage-gated Ca^{2+} channels, demonstrating an IC_{50} value of 0.3 μM in bovine adrenal chromaffin cells. The observed disruption of the voltage-gated Ca^{2+} channels most likely reflects competition between Pb and Ca^{2+} for the extracellular Ca^{2+} binding domain of the channel. Research by other laboratories supported these findings: Pb inhibited the action of multiple Ca^{2+} channels in human embryonic kidney cells transfected with L-, N-, and R-type channels (IC_{50} values of 0.38 μM , 1.31 μM , and 0.10 μM , respectively) (Peng et al., 2002) and P-type channels in cultured hippocampal neurons at concentrations up to 3 μM (Ujihara et al., 1995). However, in bovine adrenal chromaffin cells, intracellular Pb was observed to enhance Ca^{2+} currents through attenuation of the Ca^{2+} dependent deactivation of Ca^{2+} channels at an EC_{50} (half maximal effective concentration) value of 200 μM , possibly through blocking the intracellular Ca^{2+} binding domain, or through Ca^{2+} dependent dephosphorylation of the channel (Sun and Suszkiw, 1995). Recently, Pb has also been shown to enter cells (HEK293, HeLa, and PC12 cell lines) through store-operated Ca^{2+} channels (Chiu et al., 2009; Chang et al., 2008b). In particular, the Orai1-STIM1 complex was shown to be critical in the entry of Pb ions into cells, and increased Pb permeation was directly related to decreased $[\text{Ca}^{2+}]_i$ concentrations at exposure concentrations as low as 0.1 μM .

Pb also has been found to disrupt the action of Ca^{2+} -dependent K^+ channels. Alvarez et al. (1986) observed that Pb promoted the efflux of K^+ from inside-out erythrocyte vesicles in a concentration-dependent manner at concentrations of 1-300 μM , either through action on a Mg^{2+} modulatory site or through direct interaction with the Ca^{2+} binding site. Fehlau

et al. (1989) also demonstrated Pb-induced activation of the K^+ channel in erythrocytes. However, Pb only activated the K^+ channels at concentrations below 10 μM ; higher concentrations of Pb completely inhibited channel activity, indicating the modulation of K^+ permeability is due to concentration-dependent alterations in channel gating. Silken et al. (2001) observed that Pb activated K^+ channels in erythrocytes from the marine teleost *Scorpaena porcus* in a concentration-dependent manner after a 20-minute incubation; minor loss of K^+ was seen at Pb concentrations of 1-2 μM , whereas exposure to 20-50 μM Pb resulted in approximately 70% K^+ loss. Results from competitive and inhibitory binding assays suggest that Pb directly activates K^+ channels in *S. porcus*.

Disruption of Neurotransmitter Release

Pb has been shown to inhibit the evoked release of neurotransmitters by inhibiting Ca^{2+} transport through voltage-gated channels in in vitro experiments (Cooper and Manalis, 1984; Suszkiw et al., 1984). However, in these same experiments, concentrations of Pb $\geq 5 \mu M$ were also observed to actually increase the spontaneous release of neurotransmitters. Subsequent research by other groups affirmed that Pb demonstrates Ca^{2+} -mimetic properties in enhancing neurotransmitter release from cells in the absence of Ca^{2+} and Ca^{2+} -induced depolarization. Tomsig and Suszkiw (1993) reported that Pb exposure induced the release of norepinephrine (NE) from bovine adrenal chromaffin cells, and was considerably more potent (as measured by half-maximal metal-dependent release [$K_{0.5}$]) than was Ca^{2+} ($K_{0.5}$ of $4.6 \times 10^{-3} \mu M$ for Pb versus 2.4 μM for Ca^{2+}). Activation of PKC was observed to enhance the Pb-induced release of NE (Tomsig and Suszkiw, 1995). Westerink and Vijverberg (2002) observed that Pb acted as a high affinity substitute for Ca^{2+} , and triggered enhanced catecholamine release from PC12 cells at 10 μM in intact cells and 0.03 μM in permeabilized cells. The suppression of Ca^{2+} -evoked release of neurotransmitters combined with the ability of Pb to enhance spontaneous releases could result in higher noise observed in the synaptic transmission of nerve impulses in Pb-exposed animals.

In rats exposed to Pb at concentrations of 1,000–10,000 ppm in drinking water beginning at gestational days GD15-GD16 and continuing to postnatal days PND120, decreases in total K^+ -stimulated hippocampal gamma aminobutyric acid (GABA) release were seen at exposure levels of 1,000–5,000 ppm (range of mean [SD] blood Pb levels: 26.8 [1.3] - 61.8 [2.9] $\mu g/dL$) (Lasley and Gilbert, 2002). Maximal effects were observed at 2,000 ppm Pb in drinking water, but effects were less evident at 5,000 ppm and were absent at 10,000 ppm. In the absence of Ca^{2+} , K^+ -induced GABA release was increased with the two highest Pb exposure concentrations, suggesting a Pb-induced enhancement of K^+ -evoked release of GABA. The authors suggest that this pattern of response indicates that Pb is a potent suppressor of K^+ -evoked release at low concentrations, but a

Ca²⁺ mimic in regard to independently inducing exocytosis and evoking neurotransmitter release at higher concentrations ([Lasley and Gilbert, 2002](#)). Suszkiw (2004) reports that augmentation of spontaneous release of neurotransmitters may involve Pb-induced activation of CaMKII-dependent phosphorylation of synapsin I or direct activation of synaptotagmin I. Further, Suszkiw (2004) suggests that unlike the intracellularly mediated effects of Pb on spontaneous release of neurotransmitters, Pb-induced inhibition of evoked transmitter releases is largely due to extracellular blockage of the voltage-gated Ca²⁺ channels.

In summary, Pb has been shown to disrupt ion transport mechanisms in toxicological and epidemiologic studies. Specific mechanisms disrupted include various cation-specific ATPases and voltage-gated cation channels. Alterations in ion transport functions have also been shown to disrupt neurotransmitter release in both in vivo and in vitro experiments.

4.2.2.3 Displacement of Metal Ions and Perturbed Protein Function

The binding of metal ions to proteins causes specific changes in protein shape, and these conformational changes may alter specific cellular function of many proteins ([Kirberger and Yang, 2008](#)). Metal binding sites on proteins are generally ion-specific and are influenced by multiple factors, including binding geometries, ligand preferences, ionic radius, and metal coordination numbers ([Kirberger and Yang, 2008](#); [Garza et al., 2006](#)). The coordination chemistry that normally regulates metal-protein binding makes many proteins particularly susceptible to perturbation from Pb, as it is able to function with flexible coordination numbers and can bind multiple ligands ([Kirberger and Yang, 2008](#); [Garza et al., 2006](#)). However, due to differences in its physical properties, Pb induces abnormal conformational changes when it binds to proteins ([Kirberger and Yang, 2008](#); [Bitto et al., 2006](#); [Garza et al., 2006](#); [Magyar et al., 2005](#)), and these structural changes elicit altered protein function. It is known that [Ca²⁺]_i is an important second messenger in cell signaling pathways, and operates by binding directly to and activating proteins such as calmodulin and PKC ([Goldstein, 1993](#)). Alterations in the functions of both of these proteins due to direct interaction with Pb have been well documented in the literature.

PKC is a family of serine/threonine protein kinases critical for cell signaling and important for cellular processes, including growth and differentiation ([Goldstein, 1993](#)). PKC contains a “C2” Ca²⁺-binding domain and requires binding of the cation, as well as the presence of diacylglycerol and phospholipids, for proper cellular activity ([Garza et](#)

[al., 2006](#)). Markovac and Goldstein ([1988b](#)) observed that, in the absence of Ca^{2+} , exposure to 10^{-6} μM concentrations of Pb for 5 minutes directly activated PKC purified from rat brains. The activation of PKC by Pb was more potent than was Ca^{2+} -dependent activation by five orders of magnitude. Long et al. ([1994](#)) affirmed these findings, reporting that Pb had a K_{act} 4,800 times smaller than that of Ca^{2+} (5.5×10^{-5} μM versus 25 μM , following a 3 minute exposure). However, Ca^{2+} had a higher maximal activation of PKC than did Pb. This possibly indicates the presence of multiple Ca^{2+} -binding sites on the protein, and that Pb may bind the first site more efficiently than does Ca^{2+} , but not subsequent sites. Tomsig and Suszkiw ([1995](#)) further demonstrated the ability of Pb to activate PKC in bovine adrenal chromaffin cells incubated with 10^{-6} μM concentrations of Pb for 10 minutes but also reported that activation of PKC by Pb was only partial (approximately 40% of the maximum activity induced by Ca^{2+}) and tended to decrease at concentrations $>1 \times 10^{-3}$ μM .

Contrary to the above findings, Markovac and Goldstein ([1988a](#)) observed that Pb and Ca^{2+} activated PKC at equivalent concentrations and efficacies when broken cell preparations of rat brain microvessels were incubated with either cation for 45 minutes. However, when PKC activation was investigated in whole vessel preparations, no activation was observed, but PKC did become redistributed from the cytosolic to the particulate fraction after centrifugation. This suggests that Pb redistributes PKC at μM concentrations, but does not activate the protein in brain microvessels. In human erythrocytes exposed to Pb acetate for 60 minutes, the amount of PKC found in erythrocyte membranes and total PKC activity was increased at concentrations greater than 0.1 μM ([Belloni-Olivi et al., 1996](#)). The observation that neither Ca^{2+} nor diacylglycerol concentrations were increased due to Pb exposure, indicates that Pb-induced activation of PKC is due to direct interaction with the protein. Pb-induced alterations in PKC have also been observed in other tissues, including increased activity in rabbit mesenteric arteries at 10^{-6} μM concentrations of Pb ([Watts et al., 1995](#); [Chai and Webb, 1988](#)) and human erythrocytes from Pb-exposed workers (range of blood Pb levels: 5.4 to 69.3 $\mu\text{g/dL}$) ([Hwang et al., 2002](#)), and decreased activity in mouse macrophages and the rat brain cortex at μM concentrations ([Murakami et al., 1993](#); [Lison et al., 1990](#)).

Calmodulin is another important protein essential for proper Ca^{2+} -dependent cell signaling. Calmodulin contains an “EF-hand” Ca^{2+} binding domain, which is dependent on the cation for proper activity ([Garza et al., 2006](#)). Calmodulin regulates events as diverse as cellular structural integrity, gene expression, and maintenance of membrane potential ([Vetter and Leclerc, 2003](#); [Saimi and Kung, 2002](#)). Habermann et al. ([1983](#)) observed that exposure to Pb altered numerous cellular functions of calmodulin, including activation of calmodulin-dependent phosphodiesterase activity after 10 minutes

incubation (minimal activation at 0.1 μM , $\text{EC}_{50} = 0.5\text{-}1.0\ \mu\text{M}$), stimulation of brain membrane phosphorylation at Pb concentrations greater than 0.4 μM after 1 minute incubation, and increased binding of calmodulin to brain membranes at Pb concentrations greater than 1 μM after 10 minutes incubation. Habermann et al. (1983) reported that the affinity of Pb for Ca^{2+} -binding sites on calmodulin was approximate to that of Ca^{2+} itself ($K_d \sim 20\ \mu\text{M}$), whereas Richardt et al. (1986) observed that Pb was slightly more potent than Ca^{2+} was at binding calmodulin ($\text{IC}_{50} = 11$ and $26\ \mu\text{M}$, respectively). Both studies indicated that Pb was much more effective at binding to calmodulin than was any other metal cation investigated (e.g., Hg, Cd, Fe). Kern et al. (2000) observed that Pb was more potent in binding to, and affecting conformational changes in, calmodulin compared to Ca^{2+} (EC_{50} values of $4\text{-}5.5 \times 10^{-4}\ \mu\text{M}$ [threshold = $1 \times 10^{-4}\ \mu\text{M}$] and $0.45\text{-}0.5\ \mu\text{M}$ [threshold = $0.1\ \mu\text{M}$], respectively). Pb, in the absence of Ca^{2+} , was also observed to activate calmodulin-dependent cyclic nucleotide phosphodiesterase activity at much lower concentrations compared to Ca^{2+} (EC_{50} value $4.3 \times 10^{-4}\ \mu\text{M}$ [threshold = $3 \times 10^{-4}\ \mu\text{M}$] versus EC_{50} $1.2 \times 10^{-3}\ \mu\text{M}$ [threshold = $0.2\ \mu\text{M}$; 50 minute incubation]). When incubated with physiological concentrations of Ca^{2+} , Pb induced phosphodiesterase activity at concentrations as low as $5 \times 10^{-5}\ \mu\text{M}$. Pb activated calcineurin, a Ca^{2+} -dependent phosphatase with widespread distribution in the brain and immune system, at threshold concentrations as low as $2 \times 10^{-5}\ \mu\text{M}$ in the presence of Ca^{2+} (incubation time = 30 minutes), but inhibited its activity at concentrations greater than $2 \times 10^{-4}\ \mu\text{M}$ (Kern and Audesirk, 2000). Thus, $10^{-6}\ \mu\text{M}$ concentrations of intracellular Pb appear to amplify the activity of calmodulin and thus can be expected to alter intracellular Ca^{2+} signaling in exposed cells (Kern et al., 2000). Mas-Oliva (1989) observed that low-exposure ($<1\ \mu\text{M}$, 20 minute incubation) stimulatory effects of Pb exposure on the activity of $\text{Ca}^{2+}/\text{Mg}^{2+}$ ATPase was due to Pb binding to calmodulin and subsequent activation of the ion pore. Ferguson et al. (2000) observed that exposure of rat hippocampal neurons to Pb for 1 to 48 hours resulted in increased activation of a calmodulin-dependent Ca^{2+} extrusion mechanism.

Pb has also been observed to alter the activity of other proteins that rely on Ca^{2+} binding for normal cellular function. Osteocalcin is a matrix protein important in bone resorption, osteoclast differentiation, and bone growth; and has three Ca^{2+} -binding sites (Dowd et al., 2001). Incubation of osteocalcin in solution with Ca^{2+} and Pb resulted in the competitive displacement of Ca^{2+} by Pb (Dowd et al., 1994). Pb was found to bind to osteocalcin more than 1,000 times more tightly than was Ca^{2+} ($K_d = 1.6 \times 10^{-2}\ \mu\text{M}$ versus $7.0\ \mu\text{M}$, respectively), and analysis with nuclear magnetic resonance (NMR) indicated that Pb induced similar, though slightly different, secondary structures in osteocalcin, compared to Ca^{2+} . The authors hypothesized that the observed difference in Pb-bound osteocalcin structure may explain previous findings in the literature that Pb exposure reduced osteocalcin adsorption to hydroxyapatite (Dowd et al., 1994). Further research by this

group also found that Pb binded osteocalcin approximately 10,000-times more tightly than did Ca^{2+} ($K_d = 8.5 \times 10^{-2} \mu\text{M}$ versus $1.25 \times 10^3 \mu\text{M}$, respectively) ([Dowd et al., 2001](#)). However, the authors reported that Pb exposure actually caused increased hydroxyapatite adsorption at concentrations 2-3 orders of magnitude lower than that seen with Ca^{2+} . Additionally, Pb can displace Ca^{2+} in numerous other Ca^{2+} -binding proteins, such as proteins important in muscle contractions, renal Ca^{2+} transport and neurotransmission, including troponin C, parvalbumin, CaBP I and II, phospholipase A_2 , and synaptotagmin I, at concentrations as low as the $10^{-3} \mu\text{M}$ range ([Bouton et al., 2001](#); [Osterode and Ulberth, 2000](#); [Richardt et al., 1986](#)).

Pb can displace metal cations other than Ca^{2+} that are requisite for protein function. One of the most researched targets for molecular toxicity of Pb is the second enzyme in the heme synthetic pathway, aminolevulinic acid dehydratase (ALAD). ALAD contains four Zn-binding sites, and all four need to be occupied to confer full enzymatic activity ([Simons, 1995](#)). ALAD has been identified as the major protein binding target for Pb in human erythrocytes ([Bergdahl et al., 1997a](#)), and inhibition of the enzyme is found in the erythrocytes of Pb-exposed workers and adolescents in association with blood Pb levels (blood Pb levels $>10 \mu\text{g/dL}$) ([Ahamed et al., 2006](#); [Ademuyiwa et al., 2005b](#)), in lysed human erythrocytes exposed to Pb in vitro for 60 minutes ($K_i = 7 \times 10^{-6} \mu\text{M}$) ([Simons, 1995](#)), and in erythrocytes of rats exposed to 25 mg/kg Pb once a week for 4 weeks (mean [SD] blood Pb level: 6.56 [0.98] $\mu\text{g/dL}$) ([Lee et al., 2005](#)). Additional experiments indicated that lower concentrations of Zn result in greater inhibition of enzyme activity by Pb, suggesting a competitive inhibition between Zn and Pb at a single site ([Simons, 1995](#)).

Zn-binding domains are also found in transcription factors and proteins necessary for gene expression, including GATA proteins and transcription factors TFIIA, Sp1, and Erg-1 ([Ghering et al., 2005](#); [Huang et al., 2004](#); [Reddy and Zawia, 2000](#); [Hanas et al., 1999](#); [Zawia et al., 1998](#)). Pb was found to form tight complexes with the cysteine residues in GATA proteins (Pb stoichiometric stability constants of $[\text{CF}\beta_1^{\text{Pb}}] = 6.4 (\pm 2.0) \times 10^9 \text{M}^{-1}$ for single C-terminal GATA Zn finger from chicken and $[\text{DF}\beta_2^{\text{Pb}}] = 6.3 (\pm 6.3) \times 10^{19} \text{M}^{-2}$ for double-GATA Zn finger from human), and was able to displace bound Zn from the protein under physiologically relevant conditions ([Ghering et al., 2005](#)). Once Pb was bound to GATA proteins, they displayed decreased ability to bind to DNA (Pb concentrations $\geq 1.25 \mu\text{M}$) and activate transcription. Pb at a minimum concentration of $10 \mu\text{M}$ also binds to the Zn domain of TFIIA, inhibiting its ability to bind DNA ([Huang et al., 2004](#); [Hanas et al., 1999](#)). Huang et al. (2004) also reported that exposure to Pb caused the dissociation of TFIIA-DNA adducts and using NMR spectroscopy, found that altered TFIIA activity was the result of a Pb-induced abnormal protein conformation.

Pb exposure modulated the DNA-binding profiles of the transcription factors Sp1 and Erg-1 in rat pups exposed to 2,000 ppm Pb acetate via lactation, resulting in a shift in DNA-binding toward early development (i.e., the first week following birth) ([Reddy and Zawia, 2000](#); [Zawia et al., 1998](#)). The shifts in Sp1 DNA-binding profiles were shown to be associated with abnormal expression of genes related to myelin formation ([Section 4.2.7.5](#)). Further mechanistic research utilizing a synthetic peptide containing a Zn finger motif demonstrated that Pb can bind the histidine and cysteine residues of the Zn finger motif, thus displacing Zn and resulting in an increase in the DNA-binding efficiency of the synthetic peptide ([Razmiafshari et al., 2001](#); [Razmiafshari and Zawia, 2000](#)). However, in DNA-binding assays utilizing recombinant Sp1 (which has three Zn finger motifs, opposed to only one in the synthetic peptide), 37 μM Pb was the lowest concentration observed to abolish the DNA-binding capabilities of Sp1 ([Razmiafshari and Zawia, 2000](#)).

Pb has also been reported to competitively inhibit Mg binding and thus inhibit the activities of adenine and hypoxanthine/guanine phosphoribosyltransferase in erythrocyte lysates from rats exposed to 1,000 ppm Pb in drinking water for 9 months (mean [SD] blood Pb level: 7.01 [1.64] $\mu\text{g/dL}$) and in in vitro human erythrocyte lysates exposed to 0.1 μM Pb for as little as 5 minutes ([Baranowska-Bosiacka et al., 2009](#)) and also inhibit cGMP phosphodiesterase at 10^{-6} μM concentrations in homogenized bovine retinas ([Srivastava et al., 1995](#)). Pb was also reported to inhibit pyrimidine 5'-nucleotidase through competitive inhibition of Mg binding, resulting in conformational changes and improper amino acid positioning in the active site ([Bitto et al., 2006](#)).

In summary, Pb has been shown to displace metal cations from the active sites of multiple enzymes and proteins, and thus to alter the functions of those proteins in occupationally-exposed humans with blood Pb levels of 5.4-69.3 $\mu\text{g/dL}$, in adult rodents with blood Pb levels of 6.5 $\mu\text{g/dL}$ (exposure 4 weeks), in suckling rats exposed to 2,000 ppm Pb via lactation, and in cell-free and cellular in vitro experiments conducted at exposure concentrations ranging from 10^{-6} μM to 1 μM . These alterations in protein function have implications for numerous cellular and physiological processes, including cell signaling, growth and differentiation, gene expression, energy metabolism, and biosynthetic pathways. [Table 4-1](#) provides a list of enzymes and proteins whose function may be perturbed by Pb exposure.

Table 4-1 Enzymes and proteins potentially affected by exposure to Pb and the metal cation cofactors necessary for their proper physiological activity.

	Metalloprotein/Enzyme	Direction of Action^a	Metal Cation; Reference
Enzymes	Aminolevulinic acid dehydratase	↓	Zn; Simons (1995)
	Ferrochelatase	↓	Fe (2Fe-2S Cluster); Crooks et al. (2010)
	Superoxide dismutase	↓↑	Mn, Cu, Zn, Fe; Antonyuk et al. (2009), Borgstahl et al. (1992)
	Catalase	↓↑	Fe (Heme); Putnam et al. (2000)
	Glutathione peroxidase	↓↑	Se; Rotruck et al. (1973)
	Guanylate cyclase	↓	Fe (Heme); Boerrigter and Burnett (2009)
	cGMP phosphodiesterase	↓	Mg, Zn; Ke (2004)
	NAD synthase	↓	Mg; Hara et al. (2003)
	NAD(P)H oxidase	↑	Ca ²⁺ ; Leseney et al. (1999)
	Pyrimidine 5'-nucleotidase	↓	Mg, Ca ²⁺ ; Bitto et al. (2006), Amici et al. (1997), Paglia and Valentine (1975)
	Erythrocyte phosphoribosyltransferase	↓	Mg ²⁺ ; Deng et al. (2010), Arnold and Kelley (1978)
Ion Channels/Transport	ATPase	↓↑	Ca ²⁺ , Mg, Na/K; Technische Universitat Braunschweig (2011)
	Mitochondrial transmembrane pore	↑	Ca ²⁺ ; He et al. (2000)
	Calcium-dependent potassium channel	↑	Ca ²⁺ ; Silkin et al. (2001), Alvarez et al. (1986)
Signal Transduction	Protein kinase C	↓↑	Ca ²⁺ ; Garza et al. (2006)
	Calmodulin	↑	Ca ²⁺ ; Garza et al. (2006)
Pb Binding	Metallothionein	↑	Zn, Cu; Yu et al. (2009)
DNA Binding	GATA transcriptional factors	↓	Zn; Hanas et al. (1999), Huang et al. (2004)

^a↑ indicates increased activity; ↓ indicates decreased activity; ↓↑ indicates activity can be alternatively increased or decreased.

4.2.2.4 Mitochondrial Abnormality

Alterations in mitochondrial function, including disruptions in ion transport, ultrastructural changes, altered energy metabolism, and perturbed enzyme activities due to Pb exposure are well documented in the scientific literature. Exposure of rats to Pb in feed (10,000 ppm Pb for 4, 6, 8, 10, 12, or 20 weeks) or drinking water (300 ppm for 8 weeks, 500 ppm for 7 months, or 10,000 ppm Pb for 9 months) resulted in gross ultrastructural changes in renal tubule mitochondria and epididymal mitochondria characterized as a general swollen appearance with frequent rupture of the outer membrane, distorted cristae, loss of cristae, frequent inner compartment vacuolization, observation of small inclusion bodies, and fusion with adjacent mitochondria ([Wang et al., 2010e](#); [Marchlewicz et al., 2009](#); [Navarro-Moreno et al., 2009](#); [Goyer, 1968](#); [Goyer et al., 1968](#)).

Transmembrane mitochondrial ion transport mechanisms have been found to be perturbed by exposure to Pb. Pb inhibits the uptake of Ca^{2+} into mitochondria ([Parr and Harris, 1976](#)), while simultaneously stimulating the efflux of Ca^{2+} out of the organelle ([Simons, 1993a](#)), thus disrupting intracellular/mitochondrial Ca^{2+} homeostasis. Pb exposure has also been shown to decrease the mitochondrial transmembrane potential in primary cerebellar granule neuronal cultures from rats exposed to 1,000 ppm Pb in drinking water throughout gestation and lactation ([Baranowska-Bosiacka et al., 2011b](#)), astroglia incubated with 0.1 or 1.0 μM Pb for 14 days ([Legare et al., 1993](#)), proximal tubule cells exposed to 0.25, 0.5, and 1.0 μM for 12 hours ([Wang et al., 2009c](#)), and retinal rod photoreceptor cells incubated with 0.01 to 10 μM for 15 minutes ([He et al., 2000](#)). Further research indicated that Pb-induced mitochondrial swelling and decreased membrane potential is the result of the opening of a mitochondrial transmembrane pore (MTP), possibly by Pb directly binding to the metal (Ca^{2+})-binding site on the matrix side of the pore ([Bragadin et al., 2007](#); [He et al., 2000](#)). Opening of the MTP is the first step of the mitochondrial-regulated apoptotic cascade pathway in many cells ([Rana, 2008](#); [Lidsky and Schneider, 2003](#)). He et al. (2000) additionally observed other indicators of apoptosis including, cytochrome c release from mitochondria, and caspase-9 and -3 activation following exposure of retinal rod cells to Pb. Induction of mitochondrially-regulated apoptosis via stimulation of the caspase cascade following exposure to Pb has also been observed in rat hepatic oval cells ([Agarwal et al., 2009](#)).

Altered Energy Metabolism

Pb has been reported to alter normal cellular bioenergetics. In mitochondria isolated from the kidneys of rats exposed to 10,000 ppm Pb in feed for 6 weeks, the rate of oxygen uptake during ADP-activated (state 3) respiration was lower compared to controls ([Goyer](#)

[et al., 1968](#)). The rate of ATP formation from exposed mitochondria was observed to be approximately 50% that of control mitochondria. A decrease in state 3 respiration and respiratory control ratios (state 3/state 4 [succinate or pyruvate/malate-activated]) was also observed in kidney mitochondria from rats exposed continuously from conception to six or nine months of age (i.e., gestationally, lactationally, and via drinking water after weaning) to 50 or 250 ppm Pb ([Fowler et al., 1980](#)). Pb-induced decreases in ATP and adenylate energy charge (AEC) were observed concurrently with increases in ADP, AMP, and adenosine in adult rats exposed to 10,000 ppm Pb in drinking water for 9 months ([Marchlewicz et al., 2009](#)). Similarly, ATP and AEC were decreased, and AMP increased, in primary cerebellar granule neuronal cultures from rats exposed to 1,000 ppm Pb in drinking water throughout gestation and lactation ([Baranowska-Bosiacka et al., 2011b](#)). One μM Pb (48 hours) was the lowest concentration observed to decrease cellular ATP levels in NGF-differentiated PC-12 cells, and these changes were correlated with a Pb-induced decrease in the expression of the mitochondrial voltage-dependent anion channel, which maintains cellular ATP levels in neurons ([Prins et al., 2010](#)). Dowd et al. ([1990](#)) reported that oxidative phosphorylation was decreased up to 74% after exposure of osteoblasts to 10 μM Pb. Parr and Harris ([1976](#)) reported that Pb inhibited both coupled and uncoupled respiratory oxygen use in mitochondria, and that Pb prevented pyruvate, but not malate, uptake. Mitochondrial levels of ATP were diminished after Pb exposure, and the authors compared the effects of Pb on the energy supply to the actions of classic respiratory inhibitors, low temperature, and chemical uncouplers. Bragadin et al. ([1998](#)) supported this view by demonstrating that alkylated Pb compounds acted as a chemical uncoupler of respiration by abolishing the proton gradient necessary for oxidative phosphorylation. Further, the enzymatic activities of complex I and IV of the respiratory chain have been shown to be decreased in the peroneous longus muscle of rats exposed to 250 ppm Pb (or 5 ppm thallium) in drinking water for 90 days ([Méndez-Armenta et al., 2011](#)). Contrary to the above findings, Rafalowska et al. ([1996](#)) reported that, although ATP levels did decrease in the forebrain synaptosomes prepared from rats exposed to 200 ppm Pb in water for 3 months, this chronic exposure to Pb did not inhibit oxidative phosphorylation in the synaptosomal mitochondria. Similar effects with regard to the activity of the mitochondrial oxidative chain were observed in rats injected with 15 mg Pb/kg (i.p.) daily for seven days, as reported by Struzynska et al. ([1997a](#)), although ATP levels were reported to increase after exposure to Pb.

Pb has also been shown to decrease glycolysis in osteoblasts exposed to 10 μM Pb and in human erythrocytes exposed (in vitro) to 30 $\mu\text{g/dL}$ Pb ([Grabowska and Guminska, 1996](#); [Dowd et al., 1990](#)). Contrary to these findings, Antonowicz et al. ([1990](#)) observed higher levels of glycolytic enzymes in erythrocytes obtained from Pb workers directly exposed to Pb, compared to workers exposed to lower concentrations of Pb (mean blood Pb levels: 82.1 versus 39.9 $\mu\text{g/dL}$), and suggested that Pb activated anaerobic glycolysis. In

vitro exposure of human umbilical cord erythrocytes to 100-200 µg/dL Pb for 20 hours was observed to lower the cellular pools of adenine and guanine nucleotide pools, including NAD and NADPH ([Baranowska-Bosiacka and Hlynczak, 2003](#)). These decreases in nucleotide pools were accompanied by an increase in purine degradation products (adenosine, etc.). Similar decreases in cellular nucleotide pools were observed when rats were exposed to 10,000 ppm Pb in drinking water for four weeks ([Baranowska-Bosiacka and Hlynczak, 2004](#)). In erythrocytes, nucleotides are synthesized via salvage pathways such as the adenine pathway, which requires adenine phosphoribosyltransferase. The activity of this enzyme was inhibited by exposure to Pb in human and rat erythrocytes (see above for concentration and duration) ([Baranowska-Bosiacka et al., 2009](#)).

Disruptions in erythrocyte energy metabolism have been observed in adults occupationally exposed to Pb. Nikolova and Kavalzhieva ([1991](#)) reported higher ratios of ATP/ADP in Pb-exposed workers with an average duration of exposure of 8.4 years (blood Pb not reported) than in unexposed controls. Morita et al. ([1997](#)) evaluated the effect of Pb on NAD synthetase in the erythrocytes of Pb-exposed workers (mean [SD] blood Pb level: 34.6 [20.7] µg/dL) and observed an apparent concentration-dependent decrease in NAD synthetase activity with increasing blood Pb level. The blood Pb level associated with 50% inhibition of NAD synthetase, which requires a Mg^{2+} cation for activity ([Hara et al., 2003](#)), was 43 µg/dL.

Altered Heme Synthesis

Exposure to Pb is demonstrated to inhibit two key enzymes in the biosynthetic pathway of heme: porphobilinogen synthase (i.e., δ -aminolevulinic acid dehydratase), a cytoplasmic enzyme requiring Zn for enzymatic activity that condenses two molecules of aminolevulinic acid into porphobilinogen, and ferrochelatase, a mitochondrial iron (Fe)-sulfur (S) containing enzyme that incorporates Fe^{2+} into protoporphyrin IX to create heme. Farant and Wigfield ([1990, 1987](#)) observed that Pb inhibits the activity of porphobilinogen synthase in rabbit and human erythrocytes, and that the effect on the enzyme was dependent on the affinity of Pb for thiol groups at the enzyme's active site. Taketani et al. ([1985](#)) examined the activity of Pb on ferrochelatase in rat liver mitochondria and observed that 10 µM Pb (30 minute incubation) reduced NAD(P)H-dependent heme synthesis by half when ferric, but not ferrous, iron was used. Pb inhibits the insertion of Fe^{2+} into the protoporphyrin ring and instead, Zn is inserted into the ring creating Zn-protoporphyrin (ZPP). While not directly measuring the activity of ferrochelatase, numerous studies have shown that blood Pb levels are associated with increased erythrocyte ZPP levels in humans (mean blood Pb levels ranging from 21.92 to 53.63 µg/dL) ([Mohammad et al., 2008](#); [Counter et al., 2007](#); [Patil et al., 2006b](#);

[Ademuyiwa et al., 2005b](#)) and in animals (blood Pb level: 24.7 µg/dL) ([Rendón-Ramirez et al., 2007](#)).

In summary, Pb has been shown to disrupt mitochondrial function including transmembrane mitochondrial ion transport mechanisms and has been shown to perturb normal cell bioenergetics. These effects have not only been demonstrated in in vitro toxicological studies but also exposed worker populations.

4.2.3 Protein Binding

Evidence indicates that Pb binds to proteins within cells through interactions with side group moieties (e.g., thiol residues) to form inclusion bodies and can thereby potentially disrupt cellular function ([Sections 4.2.2.3 and 4.2.2.4](#)). However, some proteins are also able to bind Pb and protect against its negative effects through sequestration. The ability of Pb to bind proteins was first reported by Blackman ([1936](#)): the formation of intranuclear inclusion bodies were observed in the liver and kidneys of children exposed to high levels of Pb. Since that time, further research has been conducted into characterizing the composition of intranuclear inclusion bodies and identifying specific Pb-binding proteins.

4.2.3.1 Intranuclear and Cytoplasmic Inclusion Bodies

Goyer ([1968](#)) and Goyer et al. ([1968](#)) observed the formation of intranuclear inclusion bodies in the renal tubules of rats fed 10,000 ppm Pb in food for up to 20 weeks. The observation of inclusion bodies was accompanied by altered mitochondrial structure and reduced rates of oxidative phosphorylation. Pb has further been observed to form cytoplasmic inclusion bodies preceding the formation of the intranuclear bodies, and to be concentrated within the subsequently induced intranuclear inclusion bodies following i.p. injection, drinking water, and dietary exposures ([Navarro-Moreno et al., 2009](#); [Oskarsson and Fowler, 1985](#); [Fowler et al., 1980](#); [McLachlin et al., 1980](#); [Choie and Richter, 1972](#); [Goyer et al., 1970b](#); [Goyer et al., 1970a](#)). Inclusion bodies have also been observed in the mitochondria of kidneys and the perinuclear space in the neurons of rats exposed to 500 ppm Pb acetate in drinking water for 60 days or 7 months ([Navarro-Moreno et al., 2009](#); [Deveci, 2006](#)). Intranuclear and cytoplasmic inclusions have also been found in organs other than the kidney, including liver, lung, and glial cells ([Singh et al., 1999](#); [Goyer and Rhyne, 1973](#)). Pb found within nuclei has also been shown to bind to the nuclear membrane and histone fractions ([Sabbioni and Marafante, 1976](#)).

Upon denaturing intranuclear inclusion bodies with strong denaturing agents, Moore et al. (1973) observed that proteins included in the bodies were rich in aspartic and glutamic acid, glycine, and cysteine. Further work by Moore and Goyer (1974) characterized the protein as a 27.5 kilodalton (kDa) protein that migrates as a single band on polyacrylamide gel electrophoresis. In contrast with the findings of Moore and Goyer, Shelton and Egle (1982) identified a 32 kDa protein with an isoelectric point of 6.3 from the kidneys of rats exposed to 10,000 ppm Pb acetate in feed or 7,500 ppm in drinking water. This protein, dubbed p32/6.3, was not found in control rats, indicating that the protein was induced by Pb exposure. This finding was in agreement with studies that indicated the formation of intranuclear inclusion bodies required protein synthesis (McLachlin et al., 1980; Choie et al., 1975). In addition to its presence in kidneys of Pb-exposed animals, p32/6.3 has been observed to be present and highly conserved in the brains of rats, mice, dogs, chickens, and humans (Egle and Shelton, 1986). Exposure of neuroblastoma cells to 50 or 100 μ M Pb glutamate for 1 or 3 days increased the abundance of p32/6.3 (Klann and Shelton, 1989). Shelton et al. (1990) determined that p32/6.3 was enriched in the basal ganglia, diencephalon, hippocampus, cerebellum, brainstem, spinal cord, and cerebral cortex, and that it contained a high percentage of glycine, aspartic, and glutamic acid residues. Selvin-Testa et al. (1991) and Harry et al. (1996) reported that pre- and post-natal exposure of rats to 2,000–10,000 ppm Pb in drinking water increased the levels of another brain protein, glial fibrillary acidic protein, in developing astrocytes; and that this increase may be indicative of a demand for astrocytes to sequester Pb.

4.2.3.2 Cytosolic Pb Binding Proteins

Numerous studies have also identified cytosolic Pb-binding proteins. Two binding proteins, with molecular weights (MW) of 11.5 and 63 kDa, were identified by Oskarsson et al. (1982) in the kidney postmitochondrial cytosolic fraction prepared from adult male rats after i.p. injection with 50 mg Pb acetate/kg, followed by i.p. injection of 50 μ Ci ^{203}Pb acetate 6 days later. The two proteins were also found in the brain, but not the liver or lung. Mistry et al. (1985) identified three proteins (MW = 11.5, 63, and >200 kDa) in rat kidney cytosol, two of which, the 11.5 and 63 kDa proteins, were able to translocate into the nucleus. The 11.5 kDa kidney protein was also able to reverse Pb binding to ALAD through chelation of Pb and donation of a Zn cation to ALAD (Goering and Fowler, 1985, 1984). Cd and Zn, but not Ca^{2+} or Fe, prevented the binding of Pb to the 63 and 11.5 kDa cytosolic proteins, which agrees with previous observations that Cd is able to reduce total kidney Pb and prevent the formation of intranuclear inclusion bodies (Mistry et al., 1986; Mahaffey et al., 1981; Mahaffey and Fowler, 1977). Additional

cytosolic Pb-binding proteins have been identified in the kidneys of Pb-exposed rats and humans, including the cleavage product of α 2-microglobulin, acyl-CoA binding protein (MW = 9 kDa), and thymosin β 4 (MW = 5 kDa) ([Smith et al., 1998](#); [Fowler and DuVal, 1991](#)).

Cytosolic Pb-binding proteins distinct from kidney proteins have also been identified in the brain of Pb-exposed rats, and in human brain homogenates exposed to Pb in vitro ([Quintanilla-Vega et al., 1995](#); [DuVal and Fowler, 1989](#); [Goering et al., 1986](#)). One protein (MW = 12 kDa) was shown to alleviate hepatic ALAD inhibition due to Pb exposure through competitive binding with Pb and donation of Zn to ALAD. Cytosolic Pb-binding proteins have been shown to be high in glutamic acid, aspartic acid, and cysteine residues ([Fowler et al., 1993](#); [DuVal and Fowler, 1989](#)). Some evidence exists that cytosolic Pb-binding proteins directly target Pb and compartmentalize intracellular Pb as a protective measure against toxicity ([Qian et al., 2005](#); [Qian et al., 2000](#)).

4.2.3.3 Erythrocytic Pb Binding Proteins

The majority (94%) of Pb in whole blood is found in erythrocytes ([Ong and Lee, 1980a](#)). Originally, the major Pb-binding protein in erythrocytes was identified as hemoglobin ([Cohen et al., 2000](#); [Lolin and O'Gorman, 1988](#); [Ong and Lee, 1980a, b](#); [Raghavan and Gonick, 1977](#)). However, Bergdahl et al. ([1997b](#)) observed the principal Pb-binding protein to be 240 kDa and identified it as ALAD. Two smaller Pb-binding proteins were observed, but not identified (MW = 45 and <10 kDa). ALAD levels are inducible by Pb exposure; the total concentration of the enzyme, but not the activity, is higher in both Pb-exposed workers (blood Pb = 30-75 μ g/dL) and rats (Pb exposure = 2.5×10^{-4} μ M in drinking water) ([Boudene et al., 1984](#); [Fujita et al., 1982](#); [Fujita et al., 1981](#)).

ALAD is a polymorphic gene with three genotypes: ALAD 1-1, ALAD 1-2, or ALAD 2-2. Carriers of the ALAD-2 allele have been shown to have higher blood Pb levels than carriers of the homozygous ALAD-1 allele ([Scinicariello et al., 2007](#); [Zhao et al., 2007](#); [Kim et al., 2004](#); [Pérez-Bravo et al., 2004](#); [Smith et al., 1995a](#); [Wetmur, 1994](#); [Wetmur et al., 1991b](#); [Astrin et al., 1987](#)). Some recent studies, however, either observed lower blood Pb levels in carriers of the ALAD-2 allele or no difference in Pb levels among the different allele carriers ([Scinicariello et al., 2010](#); [Krieg et al., 2009](#); [Chen et al., 2008c](#); [Chia et al., 2007](#); [Chia et al., 2006](#); [Wananukul et al., 2006](#)).

The ALAD-2 protein binds Pb more tightly than the ALAD-1 form: in Pb-exposed workers carrying the ALAD-2 gene, 84% of blood Pb was bound to ALAD versus 81% in carriers of the ALAD-1 gene ($p = 0.03$) ([Bergdahl et al., 1997a](#)). This higher affinity for Pb in ALAD-2 carriers may sequester Pb and prevent its bioavailability for reaction

with other enzymes or cellular components. This is supported by the observation that carriers of the ALAD-2 gene have higher levels of hemoglobin ([Scinicariello et al., 2007](#)), decreased plasma levulinic acid ([Schwartz et al., 1997b](#)), decreased levels of Zn protoporphyrin ([Scinicariello et al., 2007](#); [Kim et al., 2004](#)), lower cortical bone Pb ([Smith et al., 1995b](#)), and lower amounts of dimercaptosuccinic acid (DMSA)-chelatable Pb ([Scinicariello et al., 2007](#); [Schwartz et al., 2000a](#); [Schwartz et al., 1997a](#)). However, the findings, that ALAD-2 polymorphisms reduced the bioavailability of Pb, are somewhat equivocal. Wu et al. ([2003a](#)) observed that ALAD-2 carriers had lower blood Pb level (5.8 ± 4.2 µg/dL) than carriers of the homozygous ALAD-1 gene (blood Pb level = 6.3 ± 4.1 µg/dL), and that ALAD-2 carriers demonstrated decreased renal function at lower patellar Pb concentrations than those associated with decreased renal function in homozygous ALAD-1 carriers. This potentially indicates that ALAD-2 carriers have enhanced Pb bioavailability. Weaver et al. ([2003b](#)) observed that ALAD-2 polymorphisms were associated with higher DMSA-chelatable Pb concentrations, when normalized to creatinine levels. Further, Montenegro et al. ([2006](#)) observed that compared with individuals with the ALAD 1-1 genotype, individuals with the ALAD 1-2/2-2 genotypes had a higher amount of Pb in the plasma (0.44 µg/L versus 0.89 µg/L, respectively) and in the percent plasma/blood ratio (0.48% versus 1.45%, respectively). This potentially suggests that individuals with the ALAD 1-2/2-2 genotypes are at increased risk of Pb-induced health effects due to lower amounts of Pb sequestration by erythrocyte ALAD, although this study did not specifically investigate the clinical implications of ALAD polymorphisms.

ALAD has the estimated capacity to bind Pb at 85 µg/dL in erythrocytes and 40 µg/dL in whole blood ([Bergdahl et al., 1998](#)). The 45 kDa and <10 kDa Pb-binding proteins bound approximately 12-26% and <1% of the blood Pb, respectively. At blood Pb concentrations greater than 40 µg/dL, greater binding to these components would likely be observed. Bergdahl et al. ([1998](#)) tentatively identified the 45 kDa protein as pyrimidine-5'-nucleotidase and the <10 kDa protein as acyl-CoA binding protein. Smith et al. ([1998](#)) previously identified acyl-CoA binding protein as a Pb-binding protein found in the kidney.

4.2.3.4 Metallothionein

In adults occupationally exposed to Pb, the presence of an inducible, low-molecular weight (approximately 10 kDa) Pb-binding protein was identified in multiple early studies ([Gonick et al., 1985](#); [Raghavan et al., 1981, 1980](#); [Raghavan and Gonick, 1977](#)). The presence of this low molecular weight protein seemed to have a protective effect, as workers who exhibited toxicity at lower blood Pb concentrations were observed to have

lowered expression of this protein or low levels of Pb bound to it ([Raghavan et al., 1981, 1980](#)). The presence of low molecular weight Pb-binding proteins in exposed workers was corroborated by Lolin and O’Gorman ([1988](#)) and Church et al. ([1993a, b](#)). Further Lolin and O’Gorman ([1988](#)) reported that the observed protein was only present when blood Pb levels were greater than 39 µg/dL, in agreement with the Pb-binding capacity of ALAD, identified by Bergdahl et al. ([1998](#)). Xie et al. ([1998](#)) confirmed this, observing the presence of a second low molecular weight protein with greater affinity than ALAD, only at higher blood Pb levels. Church et al. ([1993a, b](#)) observed the presence of a 6-7 kDa protein in the blood of two Pb workers (blood Pb >160 µg/dL); approximately 67% of Pb was bound to the protein in the blood of the asymptomatic worker, whereas only 22% of the Pb was bound to it in the symptomatic (tremor, ataxia, extremity numbness) worker. The reported protein was rich in cysteine residues and tentatively identified as metallothionein.

Metallothionein is a low-MW metal-binding protein, most often binding Zn or Cu, that is rich in cysteine residues and plays an important role in protecting against heavy metal toxicity, maintaining trace element homeostasis, and scavenging free radicals ([Yu et al., 2009](#)). Exposure to Pb acetate induced the production of Pb- and Zn-metalllothionein in mice treated via i.p. or intravenous (i.v.) injection at 30 mg/kg ([Maitani et al., 1986](#)), in mice treated via i.p. injection at 300 µmol/kg ([Yu et al., 2009](#)), or in rats treated via i.p. injection at 24 µmol/100g ([Ikebuchi et al., 1986](#)). The induced Pb-metalllothionein consisted of 28% half-cysteine and reacted with an antibody for Zn-metalllothionein II ([Ikebuchi et al., 1986](#)). In contrast, exposure of rats to Pb via drinking water (200 or 300 ppm) failed to induce metallothionein in the kidneys or intestines ([Wang et al., 2009b](#); [Jamieson et al., 2007](#)). Goering and Fowler ([1987a, b](#)) observed that pretreatment of rats with Zn before injection with Pb resulted in Pb and Zn co-eluting with Zn-thionein, and that Zn-thionein I and II were able to bind Pb in vitro ([Goering and Fowler, 1987a, b](#)). Further, Goering and Fowler ([1987a](#)) found that kidney and liver Zn-thionein decreased binding of Pb to liver ALAD and was able to donate Zn to ALAD, thus attenuating the inhibition of ALAD due to Pb exposure. These findings are in agreement with Goering et al. ([1986](#)) and DuVal and Fowler ([1989](#)) who demonstrated that rat brain Pb-binding proteins attenuated Pb-induced inhibition of ALAD.

Metallothionein has been reported to be important in the amelioration of Pb-induced toxicity effects. Liu et al. ([1991](#)) reported that Zn-metalllothionein reduced Pb-induced membrane leakage and loss of K⁺ in cultured hepatocytes incubated with 600-3,600 µM Pb. Metallothionein-null mice exposed to 1,000, 2,000, or 4,000 ppm Pb for 20 weeks suffered renal toxicity described as nephromegaly and decreased renal function compared to Pb-treated wild-type mice ([Qu et al., 2002](#)). Interestingly, metallothionein-null mice were unable to form intranuclear inclusion bodies and accumulated less renal Pb than did

the wild-type mice ([Qu et al., 2002](#)). Increased metallothionein levels were induced by Pb exposure in non-null mice. Exposure to Pb (1,000, 2,000, or 4,000 ppm), both for 104 weeks as adults and from GD8 to early adulthood, resulted in increased preneoplastic lesions and carcinogenicity in the testes, bladder, and kidneys of metallothionein-null rats compared to wild type mice ([Tokar et al., 2010](#); [Waalkes et al., 2004](#)). Inclusion bodies were not observed in null mice. The authors concluded that metallothionein is important in the formation of inclusion bodies and in the mitigation of Pb-induced toxic effects, and that those with polymorphisms in metallothionein coding genes that are associated with reduced inducibility may be at greater susceptibility to Pb. In support of this theory, Chen et al. ([2010a](#)) observed that Pb-exposed workers with a mutant metallothionein allele had higher blood Pb levels than did homozygous carriers of the normal allele (means 24.17 and 21.27 versus 17.03 $\mu\text{g/dL}$), and had larger blood Pb-associated changes in systolic BP and serum renal function parameters.

In summary, a number of proteins have been identified that can bind and sequester Pb including ALAD and metallothionein. Additionally, evidence suggests that certain polymorphisms that alter the binding capability or inducibility of these proteins can increase the risk of Pb-induced health effects.

4.2.4 Oxidative Stress

Oxidative stress occurs when free radicals or reactive oxygen species (ROS) exceed the capacity of antioxidant defense mechanisms. This oxidative imbalance results in uncontained ROS, such as superoxide (O_2^-), hydroxyl radical (OH), and hydrogen peroxide (H_2O_2), which can attack and denature functional/structural molecules and, thereby, promote tissue damage, cytotoxicity, and dysfunction. Pb exposure has been shown to cause oxidative damage to the heart, liver, kidney, reproductive organs, brain, and erythrocytes, which may be responsible for a number of Pb-induced health effects ([Salawu et al., 2009](#); [Shan et al., 2009](#); [Vaziri, 2008b](#); [Gonick et al., 1997](#); [Sandhir and Gill, 1995](#); [Khalil-Manesh et al., 1994](#); [Khalil-Manesh et al., 1992a](#)). The origin of ROS produced after Pb exposure is likely a multipathway process, resulting from oxidation of δ -aminolevulinic acid (ALA), membrane and lipid oxidation, NAD(P)H oxidase activation, and antioxidant enzyme depletion, as discussed below. Some of these processes result from the disruption of functional metal ions within oxidative stress enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). Interestingly, Pb exposure in many species of plants, invertebrates, and vertebrates discussed in [Chapter 6](#) (*Ecological Effects of Lead*) results in upregulation of antioxidant enzymes and increased lipid peroxidation. Oxidative stress is a common mode of action for a number of other metals (e.g., Cd, Mn, As, Co, Cr) that are often

found with Pb and by which possible interactions with Pb have been suggested to occur ([Jomova and Valko, 2011](#); [Jomova et al., 2011](#); [Matović et al., 2011](#); [HaMai and Bondy, 2004](#)). Not all of these co-occurring metals directly produce ROS or redox cycle, but instead may suppress the free radical scavenging ability of the organism thus leading to oxidative stress.

4.2.4.1 δ -ALA Oxidation

The majority of Pb present in the blood accumulates in erythrocytes where it enters through passive carrier-mediated mechanisms including a vanadate-sensitive Ca^{2+} pump. Once Pb enters erythrocytes, it is predominantly found in the protein-bound form, with hemoglobin and δ -ALAD both identified as targets ([Bergdahl et al., 1997a](#)). Through its sulfhydryl and metal ion disrupting properties, Pb incorporates with and inhibits a number of enzymes in the heme biosynthetic process, including δ -ALA synthetase, δ -ALAD, and ferrochelatase. Pb has been shown to be able to disrupt the Zn ions requisite for the activity of δ -ALAD, the rate limiting step in heme synthesis, leading to enzyme inhibition at 10^{-6} μM concentrations ([Simons, 1995](#)). Additionally, blood Pb levels (mean: 7 $\mu\text{g/dL}$) have been associated with inhibited activity of δ -ALAD in humans, and the correlation was found in the group with blood Pb levels > 5 $\mu\text{g/dL}$ ([Ahamed et al., 2005](#); [Sakai and Morita, 1996](#)). A negative correlation ($r = -0.6$) was found between blood Pb levels in adolescents (range of blood Pb levels: 4 to 20 $\mu\text{g/dL}$) and blood δ -ALAD activity ([Ahamed et al., 2006](#)). This inhibition of δ -ALAD results in the accumulation of δ -ALA in blood and urine, where δ -ALA undergoes tautomerization and autoxidation. Oxidized δ -ALA generates ROS through reduction of ferricytochrome c and electron transfer from oxyHb, methHb, and other ferric and ferrous iron complexes ([Hermes-Lima et al., 1991](#); [Monteiro et al., 1991](#)). The autoxidation of δ -ALA produces O_2^- , OH, H_2O_2 , and an ALA radical ([Monteiro et al., 1989](#); [Monteiro et al., 1986](#)).

4.2.4.2 Membrane and Lipid Peroxidation

A large number of studies in humans and experimental animals have indicated that exposure to Pb can lead to membrane and lipid peroxidation. It is possible that ROS produced from δ -ALA oxidation, as described above, interacts with and disrupts membrane lipids ([Oteiza et al., 1995](#); [Bechara et al., 1993](#)). Additionally, Pb has the capacity to stimulate ferrous ion initiated membrane lipid peroxidation serving as a catalyst for these events ([Adonaylo and Oteiza, 1999](#); [Quinlan et al., 1988](#)). The extent of peroxidation of lipids varies based on the number of double bonds present in unsaturated fatty acids, since double bonds weaken the C-H bonds on the adjacent carbon, making

hydrogen (H) removal easier ([Yiin and Lin, 1995](#)). After the essential unsaturated fatty acid solutions were incubated with Pb (4-12 µg/dL, 24 hours), the production of malondialdehyde (MDA), a marker of oxidative stress and lipid oxidation end product, increased relative to the number of double bonds of the fatty acid ([Yiin and Lin, 1995](#)). In the absence of Fe²⁺, Pb has not been shown to promote lipid peroxidation; however, it may accelerate peroxidation by H₂O₂ ([Quinlan et al., 1988](#)). This could be due to altering membrane structure, restricting phospholipid movement, and facilitating the propagation of peroxidation.

Pb induces changes in the fatty acid composition of a membrane, which could lead to oxidative damage. Exposure to Pb (>62.5 ppm in drinking water, 3 weeks) in chicks promoted an increase in arachidonic acid (20:4) as a percentage of total fatty acids, and decreased the relative proportion of shorter chain fatty acids (linoleic acid, 18:2) ([Lawton and Donaldson, 1991](#)). It is possible that Pb depressed the desaturation of saturated fatty acids to the corresponding monoenoic fatty acids, while stimulating elongation and desaturation of linoleic acid to arachidonic acid. Since fatty acid chain length and unsaturation are related to the oxidative potential, changes in fatty acid membrane composition may result in enhanced lipid peroxidation. In addition, changes in fatty acids, thus membrane composition, can result in altered membrane fluidity ([Donaldson and Knowles, 1993](#)). Changes in membrane fluidity will disturb the conformation of the active sites of membrane associated enzymes, disrupt metabolic regulation, and alter membrane permeability and function.

A number of recent studies report increased measures of lipid peroxidation in various organs, tissues, and species in association with Pb. Occupational Pb exposure resulting in elevated blood Pb levels (means >8 µg/dL) reported in various countries provides evidence of lipid peroxidation, including increased plasma MDA levels ([Ergurhan-Ilhan et al., 2008](#); [Khan et al., 2008](#); [Mohammad et al., 2008](#); [Quintanar-Escorza et al., 2007](#); [Patil et al., 2006a](#); [Patil et al., 2006b](#)). One study found a correlation between the MDA levels and blood Pb levels even in the unexposed workers, although they had blood Pb levels higher than the mean blood Pb level of the current U.S. population (i.e., <12 µg/dL) ([Quintanar-Escorza et al., 2007](#)). Other studies found evidence of increased lipid peroxidation among the general population, including children, with elevated blood Pb levels (means >10 µg/dL) ([Ahamed et al., 2008](#); [Ahamed et al., 2006](#); [Jin et al., 2006](#)). In adolescents, Ahamed et al. (2006) found blood MDA levels to be positively correlated (r = 0.7) with concurrent blood Pb levels ranging between 4 and 20 µg/dL. Similar results have been shown after Pb exposure in animal studies ([Abdel Moneim et al., 2011b](#); [Pandya et al., 2010](#); [Dogru et al., 2008](#); [Yu et al., 2008](#); [Adegbesan and Adenuga, 2007](#); [Lee et al., 2005](#)). Enhanced lipid peroxidation has been found in Pb treated (50 µg, 1-4 hours) rat brain homogenates ([Rehman et al., 1995](#)), rat proximal

tubule cells (0.5-1 μ M, 12 hours) ([Wang et al., 2011b](#)), and in specific brain regions, hippocampus and cerebellum, after Pb exposure (500 ppm, 8 weeks) to rats ([Bennet et al., 2007](#)). Overall, there was a correlation between the blood Pb level and measures of lipid peroxidation often measured by MDA levels.

In summary, studies in humans and animals provide evidence for increased lipid and membrane oxidation following Pb exposure. Interestingly, many species of plants, invertebrates, and other vertebrates also exhibit increased lipid peroxidation with Pb exposure ([Sections 6.3.12.6](#) and [6.4.12.6](#)). The increase in lipid peroxidation following Pb exposure observed across species and kingdoms demonstrate an evolutionarily conserved oxidative response following Pb exposure.

4.2.4.3 NAD(P)H Oxidase Activation

NAD(P)H oxidase is a membrane bound enzyme that requires Ca^{2+} in order to catalyze the production of O_2^- from NAD(P)H and molecular oxygen ([Leseney et al., 1999](#)). Two studies provide evidence for increased activation of NAD(P)H oxidase that may contribute to the production of ROS after Pb exposure ([Ni et al., 2004](#); [Vaziri et al., 2003](#)). Vaziri et al. (2003) found increased protein expression of the NAD(P)H subunit gp⁹¹phox in the brain, heart, and renal cortex of Pb-treated rats (100 ppm in drinking water, 12 weeks). This upregulation was present in Pb-treated (1-10 ppm) human coronary artery endothelial cells, but not vascular smooth muscle cells (VSMC), which do not express the protein ([Ni et al., 2004](#)). It is possible that NAD(P)H oxidase serves as a potential source of ROS in cells that express this protein.

4.2.4.4 Antioxidant Enzyme Disruption

Oxidative stress can result not only from the increased production of ROS, but also from the decreased activity of antioxidant defense enzymes. Pb has been shown to alter the function of several antioxidant enzymes, including SOD, CAT, glucose-6-phosphate dehydrogenase (G6PD), and the enzymes involved in glutathione metabolism, GPx, glutathione-S-transferase (GST), and glutathione reductase (GR). These changes in the antioxidant defense system could be due to the high affinity of Pb for sulfhydryl groups contained within proteins and its metal ion mimicry. However changes could also be a consequence of increased oxidative damage by Pb.

Studies of the effects of Pb exposure on the activities of SOD and CAT give divergent results. These metalloprotein enzymes require various essential trace elements for proper structure and function, making them a target for Pb toxicity. CAT is a heme containing

protein that requires Fe ions to function ([Putnam et al., 2000](#)). SOD exists in multiple isoforms that require Cu and Zn (SOD1 and SOD3) ([Antonyuk et al., 2009](#)) or Mn (SOD2) ([Borgstahl et al., 1992](#)). A number of studies have found decreased activity of these enzymes ([Conterato et al., 2013](#); [Pandya et al., 2010](#); [Ergurhan-Ilhan et al., 2008](#); [Mohammad et al., 2008](#); [Yu et al., 2008](#); [Patil et al., 2006a](#); [Patil et al., 2006b](#)), whereas others have observed increased activity following Pb exposure ([Ahamed et al., 2008](#); [Lee et al., 2005](#)). The heterogeneity in species examined, (i.e., humans, rodents, boars), and Pb exposure duration and metrics may account for some of the heterogeneity in reported results. These heterogeneities in study designs limit the evaluation of whether a nonlinear concentration-response relationship could explain the conflicting results regarding the directionality of effects observed. Pb exposure (500 ppm Pb acetate, 1, 4, and 8 weeks) in adult male rats showed that SOD and CAT activity responded differently depending on the brain region analyzed and length of exposure ([Bennet et al., 2007](#)). Another study found that the brain had consistently decreased SOD activity, irrespective of Pb dose in prenatally-exposed animals (0.3 and 3.0 ppm, blood Pb level 20.4 and 24.5 µg/dL); however, hepatic SOD activity increased at low level Pb administration and decreased after high level exposure ([Uzbekov et al., 2007](#)). It is possible that the increased activity of the SOD and CAT proteins is due to activation by ROS, while decreased enzyme activity is the result of metal ion substitution by Pb, causing enzyme inactivation.

Glutathione is a tripeptide antioxidant containing a cysteine with a reactive thiol group that can act nonenzymatically as a direct antioxidant or as a cofactor in enzymatic detoxification reactions by GST. Glutathione will donate an electron while in its reduced state (GSH), which leads to conversion to the oxidized form, glutathione disulfide (GSSG). Pb binds to the thiol and can both interfere with the antioxidant capacity of GSH, and can decrease levels of GSH. Short-term administration of Pb in vitro (0.1 µM) and biomarkers of Pb exposure in humans (18 µg/dL mean blood Pb level) have been associated with decreased blood and organ GSH and cysteine content, which may be due to increased GSH efflux from tissues ([Pandya et al., 2010](#); [Pillai et al., 2010](#); [Ahamed et al., 2009](#); [Ahamed et al., 2008](#); [Flora et al., 2007](#); [Ahamed et al., 2005](#); [Chetty et al., 2005](#); [Nakagawa, 1991, 1989](#)). Long-term Pb exposure has elicited a compensatory upregulation in the biosynthesis of GSH in the attempt to overcome Pb toxicity, thus often manifesting as an increase in Pb-induced GSH in animals ([Daggett et al., 1998](#); [Corongiu and Milia, 1982](#); [Hsu, 1981](#)) and occupationally exposed adults ([Conterato et al., 2013](#); [SRC, 2002](#)). However, other studies have found that long-term Pb exposure, resulting in mean blood Pb levels between 6.6 and 22 µg/dL, causes the depletion of GSH in animals ([Lee et al., 2005](#); [Ercal et al., 1996](#)) and occupationally exposed adults ([Mohammad et al., 2008](#)). Thus, the duration of Pb exposure is important to consider when measuring GSH levels.

Glutathione reductase is able to reduce GSSG back to GSH. Therefore, an increased GSSG/GSH ratio is considered to be indicative of oxidative stress. Epidemiologic studies have found higher blood Pb levels to be associated with increases in the GSSG/GSH ratio ([Mohammad et al., 2008](#); [Ercal et al., 1996](#); [Sandhir and Gill, 1995](#)). In one study, this association was observed in a population of children with a mean blood Pb level below 10 µg/dL ([Diouf et al., 2006](#)). Studies have found mixed effects on GR activation. GR possesses a disulfide at its active site that is a target for inhibition by Pb. Studies in animals and cells have reported decreased ([Bokara et al., 2009](#); [Sandhir and Gill, 1995](#); [Sandhir et al., 1994](#)), increased ([Sobekova et al., 2009](#); [Howard, 1974](#)), and no change ([Hsu, 1981](#)) in GR activity after Pb exposure. This could be because the effect of Pb on GR varies depending on sex ([Sobekova et al., 2009](#)), and organ or organ region ([Bokara et al., 2009](#)). Additionally, heterogeneity in species examined, (i.e., humans, rodents), and Pb exposure duration and metrics may account for some of the heterogeneity in reported results. These heterogeneities in study designs make it difficult to compare exposures and doses across studies and limit the evaluation of whether a nonlinear concentration-response relationship could explain the conflicting results regarding the directionality of effects observed.

GSH is used as a cofactor for peroxide reduction and detoxification of xenobiotics by the enzymes GPx and GST. GPx requires Se (selenium) for peroxide decomposition ([Rotruck et al., 1973](#)), whereas GST functions via a sulfhydryl group. Evidence indicates that by reducing the uptake of Se, depleting cellular GSH, and disrupting protein thiols, Pb decreases the activity of GPx and GST ([Pillai et al., 2010](#); [Yu et al., 2008](#); [Lee et al., 2005](#); [Nakagawa, 1991](#); [Schrauzer, 1987](#)). Similar to other antioxidant enzymes, compensatory upregulation of these enzymes was observed after Pb exposure in animals and in Pb-exposed workers (painters with a mean blood Pb level of 5.4 µg/dL ([Conterato et al., 2013](#); [Bokara et al., 2009](#); [Ergurhan-Ilhan et al., 2008](#); [Conterato et al., 2007](#); [Daggett et al., 1998](#)). However, in another study, these enzymes were not able to compensate for the increased Pb-induced ROS, further contributing to the oxidative environment ([Farmand et al., 2005](#)).

Recently, γ -glutamyltransferase (GGT) within its normal range has been regarded as an early and sensitive marker of oxidative stress. This may be because cellular GGT metabolizes extracellular GSH to be used in intracellular GSH synthesis. Thus, cellular GGT acts as an antioxidant enzyme by increasing the intracellular GSH pool. However, the reasons for the association between GGT and oxidative stress have not been fully realized ([Lee et al., 2004](#)). In one study, occupational Pb exposure (mean blood Pb level of 29.1 µg/dL) was associated with increased serum GGT levels ([Khan et al., 2008](#)). Interestingly, higher blood Pb level was similarly associated with higher serum GGT levels in a sample of the U.S. adult population (NHANES III) ([Lee et al., 2006a](#)). In this

study of nonoccupationally-exposed individuals, a concentration-dependent relationship was observed with blood Pb levels <7 µg/dL.

In summary, Pb has been shown to alter the function of several antioxidant enzymes, including SOD, CAT, G6PD, and the enzymes involved in glutathione metabolism, GPx, GST, and GR in human populations and experimental animal models, although not consistently in the same direction. Interestingly, many species of plants, invertebrates, and other vertebrates also exhibit altered antioxidant enzyme activity following Pb exposure ([Sections 6.3.12.6, 6.4.12.6, and 6.4.21.6](#)). Alteration of these enzymes may lead to further oxidative stress following Pb exposure.

4.2.4.5 Nitric Oxide Signaling

NO (nitric oxide or nitric oxide radical), also known as endothelium-derived relaxing factor, is a potent endogenous signaling molecule involved in vasodilation. Short- and long-term Pb exposures in animals have been found to decrease the biologically active NO, not through reduction in NO-production capacity ([Vaziri and Ding, 2001](#); [Vaziri et al., 1999a](#)), but as a result of inactivation and sequestration of NO by ROS ([Malvezzi et al., 2001](#); [Vaziri et al., 1999b](#)). Endogenous NO can interact with ROS, specifically O_2^- , produced following exposure to Pb to form the highly cytotoxic reactive nitrogen species, peroxynitrite (ONOO⁻). This reactive compound can damage cellular DNA and proteins, resulting in the formation of nitrotyrosine among other products. Overabundance of nitrotyrosine in plasma and tissues is present after exposure to Pb ([Vaziri et al., 1999b](#)). NO is also produced by macrophages in the defense against certain infectious agents, including bacteria. Studies have indicated that Pb exposure can significantly reduce production of NO in human ([Pineda-Zavaleta et al., 2004](#)) and animal ([Lee et al., 2001b](#); [Tian and Lawrence, 1995](#)) immune cells, possibly leading to reduced host resistance ([Tian and Lawrence, 1996](#)).

Production of NO is catalyzed by a family of enzymes called nitric oxide synthases (NOS), including endothelial NOS (eNOS), neuronal NOS (nNOS), and inducible NOS (iNOS), which require a heme prosthetic group and a Zn cation for enzymatic activity ([Messerschmidt et al., 2001](#)). Paradoxically, the reduction in NO availability in vascular tissue following Pb exposure is accompanied by statistically significant upregulation in NOS isotypes ([Vaziri and Ding, 2001](#); [Vaziri et al., 1999a](#); [Gonick et al., 1997](#)). A direct inhibitory action of Pb on NOS enzymatic activity has been rejected ([Vaziri et al., 1999a](#)). Instead, the upregulation of NOS occurs as compensation for the decreased NO resulting from ROS inactivation ([Vaziri et al., 2005](#); [Vaziri and Ding, 2001](#); [Vaziri and Wang, 1999](#)).

Soluble Guanylate Synthase

Many biological actions of NO, such as vasorelaxation, are mediated by cyclic guanosine monophosphate (cGMP), which is produced by soluble guanylate cyclase (sGC) from the substrate guanosine triphosphate. Soluble guanylate cyclase is a heterodimer requiring one molecule of heme for enzymatic activity ([Boerrigter and Burnett, 2009](#)). In VSMC, sGC serves as the NO receptor. Marked reduction in plasma concentrations and urinary excretion of cGMP is observed after Pb exposure to rats [5 ppm Pb in drinking water for 30 days ([Marques et al., 2001](#)) and 100 ppm Pb acetate in drinking water for 3 months, resulting in a mean blood Pb level of 29.4 µg/dL ([Khalil-Manesh et al., 1993b](#))] ([Marques et al., 2001](#); [Khalil-Manesh et al., 1993b](#)). In addition, Pb exposure downregulated the protein abundance of sGC in vascular tissue ([Farmand et al., 2005](#); [Courtois et al., 2003](#); [Marques et al., 2001](#)). This downregulation in sGC was prevented by antioxidant therapy (ascorbic acid) suggesting that oxidative stress also plays a role in Pb-induced downregulation of sGC (no change in blood Pb level was observed after ascorbic acid treatment) ([Marques et al., 2001](#)).

4.2.5 Inflammation

Misregulated inflammation represents one of the major hallmarks of Pb-induced immune effects. It is important to note that this can manifest in any tissue where immune cell mobilization and tissue insult occurs. Enhanced inflammation and tissue damage occurs through the modulation of inflammatory cell function and production of pro-inflammatory cytokines and metabolites. Overproduction of ROS and an apparent depletion of antioxidant protective enzymes and factors (e.g., Se) accompany this immunomodulation ([Chetty et al., 2005](#)).

Traditional immune-mediated inflammation can be seen with bronchial hyperresponsiveness, asthma, and respiratory infections, some of which have been associated with exposure to Pb. But it is important to recognize that any tissue or organ can be affected by immune-mediated inflammatory dysfunction given the distribution of immune cells as both permanent residents and infiltrating cell populations ([Mudipalli, 2007](#); [Carmignani et al., 2000](#)). Pb has been associated with multiple indicators of inflammation in multiple cell types. Pb has also induced renal tubulointerstitial inflammation (100 ppm exposure for 14 weeks) ([Rodriguez-Iturbe et al., 2005](#)) (24.6 µg/dL blood Pb level, 150 ppm for 16 weeks) ([Roncal et al., 2007](#)). Renal tubulointerstitial inflammation has been coupled with activation of the redox sensitive nuclear transcription factor kappa B (NFκB) and lymphocyte and macrophage infiltration in rats (100 ppm for 14 weeks resulting in mean blood Pb levels ranging 23.7 µg/dL)

([Bravo et al., 2007](#)). These events could occur in response to the oxidative environment arising from Pb exposure, since Pb-induced inflammation and NFκB activation can be ameliorated by antioxidant therapy ([Rodriguez-Iturbe et al., 2004](#)). Pb spheres implanted in the brains of rats produced neutrophil-driven inflammation with apoptosis and indications of neurodegeneration ([Nakao et al., 2010](#)).

Inflammation can be mediated by the production of chemical messengers such as prostaglandins (PG). Pb exposure has been associated with increased arachidonic acid (AA) metabolism, thus elevating the production of PGE₂, PGF₂, and thromboxane in occupationally-exposed humans (mean blood Pb level 48 µg/dL) ([Cardenas et al., 1993](#)) and animal and cell models (e.g., 0.01 µM, 48 hours) ([Chetty et al., 2005](#); [Flohé et al., 2002](#); [Knowles and Donaldson, 1997](#); [Lee and Battles, 1994](#)). Dietary Pb exposure of animals (500 ppm, 19 days) can increase the percentage of cell membrane AA, the precursor of cyclooxygenase and lipoxygenase metabolism to PGs and leukotrienes ([Knowles and Donaldson, 1990](#)). Additionally, Pb (1 µM) may promote the release of AA via activation of phospholipase A₂, as shown in isolated VSMC ([Dorman and Freeman, 2002](#)).

Inflammation may be the result of increased pro-inflammatory signaling or may stimulate these signaling pathways. Pb can elevate the expression of the pro-inflammatory transcription factors NFκB and activator protein-1 (AP-1), as well as the AP-1 component c-Jun ([Korashy and El-Kadi, 2008](#); [Korashy and Ei-Kadi, 2008](#); [Bravo et al., 2007](#); [Ramesh et al., 1999](#); [Pyatt et al., 1996](#)). Pb exposure (25 µM) to dendritic cells stimulated phosphorylation of the Erk/MAPK pathway, but not p38, STAT3 or 5, or CREB ([Gao et al., 2007](#))

4.2.5.1 Cytokine Production

There are three modes by which Pb has been shown to affect immune cytokine production. First, Pb can act on macrophages to elevate the production of pro-inflammatory cytokines such as TNF-α and interleukin (IL)-6 ([Cheng et al., 2006](#); [Chen et al., 1999](#); [Miller et al., 1998](#); [Dentener et al., 1989](#)). This can result in local tissue damage during the course of immune responses affecting such targets as the liver. Second, Pb can skew the ratio of IL-12/IL-10 such that T-derived lymphocyte helper (Th)1 responses are suppressed and Th2 responses are promoted ([Gao et al., 2007](#)) possibly by affecting dendritic cells. Third, when acquired immune responses occur following exposure to Pb, Th1 lymphocyte production of cytokines is suppressed (e.g., IFN-γ) ([Lynes et al., 2006](#); [Heo et al., 1996](#)); in contrast, Th2 cytokines such as IL-4, IL-5, and IL-6 are elevated ([Gao et al., 2007](#); [Chen et al., 2004](#); [Kim and Lawrence,](#)

[2000](#)). The combination of these three modes of cytokine changes induced by Pb can create a hyperinflammatory state among innate immune cells and skew acquired immunity toward Th2 responses.

Iavicoli et al. ([2006b](#)) reported that low blood Pb concentrations produced significant changes in cytokine levels in mice. At a low dietary Pb concentration (0.11 ppm, blood Pb level of 1.6 µg/dL), IL-2 and IFN-γ were decreased compared to the controls (0.02 ppm, 0.8 µg/dL), indicating a suppressed Th1 response. As the dietary and blood Pb concentrations increased (resulting in blood Pb levels 12-61 µg/dL), a Th2 phenotype was observed with suppressed IFN-γ and IL-2 and elevated IL-4 production. These findings support the notion that the immune system is remarkably sensitive to Pb-induced functional alterations and that nonlinear effects may occur at low Pb exposures. TGF-β production was also altered by Pb exposure to transfected mouse limb bud mesenchymal stem cells (1 µM, 3 days) ([Zuscik et al., 2007](#)). IL-2 is one of the more variable cytokines with respect to Pb-induced changes. Depending upon the protocol it can be slightly elevated in production or unchanged. Recently, Gao et al. ([2007](#)) found that Pb-treated dendritic cells (25 µM) promoted a slight but statistically significant increase in IL-2 production among lymphocytes. Proinflammatory cytokines have been measured in other organs and cell types after Pb exposure. Elevation of IL-1β and TNF-α were observed in the hippocampus of rats after Pb treatment (15 ppm, i.p., daily for 2 weeks, blood Pb level of 30.8 µg/dL) and increased IL-6 was found in the forebrain ([Strużyńska et al., 2007](#)).

Consistent with animal studies, epidemiologic studies also found higher concurrent blood Pb levels in children and occupationally-exposed adults to be associated with a shift toward production of Th2 cytokines relative to Th1 cytokines. The evidence in children was based on comparisons of serum cytokine levels among groups with different blood Pb levels without consideration of potential confounding factors. Among children ages 9 months to 6 years in Missouri, Lutz et al. ([1999](#)) found that children with concurrent blood Pb levels 15-19 µg/dL had higher serum levels of IL-4 and IgE ([Section 4.6.3](#)) than did children with lower blood Pb levels. These results were consistent with the mode of action for IL-4 to activate B cells to induce B cell class switching to IgE. In another study, concurrent blood Pb levels did not differ by residence in old versus new homes or by urban versus rural residence (means: 3.2-3.8 µg/dL) but were higher among children living near an oil refinery, in particular, among children with known respiratory allergies (mean: 8.8 µg/dL) ([Hsiao et al., 2011](#)). This latter group of children also had the lowest serum levels of IFN-γ and highest levels of IL-4. There was no direct comparison of cytokine levels between blood Pb level groups in the population overall; however, cytokine levels were similar between healthy and allergy groups in the other Pb source groups that had similar blood Pb levels. Thus, the differences in cytokine levels between

healthy and allergic children living near the oil refinery may have been influenced by differences in their blood Pb levels or other factors related to residence near an oil refinery.

Evidence of association between blood Pb levels and cytokine levels in nonoccupationally-exposed adults was unclear. Among healthy adult university students in Incheon, Korea, Kim et al. ([2007](#)) found associations of concurrent blood Pb level with serum levels of TNF- α and IL-6 that were larger among male students with blood Pb levels 2.51-10.47 $\mu\text{g/dL}$. Notably, the relative contributions of lower recent versus higher past Pb exposures to these cytokine effects is not known. In models that adjusted for age, sex, BMI, and smoking status, a 1 $\mu\text{g/dL}$ increase in blood Pb level was associated with a 23% increase (95% CI: 4, 55%) in log of TNF- α and a 26% increase (95% CI: 0, 55%) in log of IL-6. The association between levels of blood Pb and plasma TNF- α was greater among men who were GSTM1 null or had the TNF- α GG genotype. For the association between blood Pb level and plasma IL-6, the effect estimate was slightly elevated in TNF- α GG genotype but not elevated in the GSTM1 positive group. The effects of Pb on several physiological systems have been hypothesized to be mediated by the generation of ROS ([Daggett et al., 1998](#)). Thus, the null variant of GSTM1, which is associated with reduced elimination of ROS, may increase the risk of Pb-associated immune effects. The results for the TNF- α polymorphism are difficult to interpret. The GG genotype is associated with lower expression of TNF- α , and the literature is mixed with respect to which variant increases risk of inflammation-related conditions. A study of adults in Italy did not provide quantitative results and only reported a lack of statistically significant correlation between blood Pb levels and Th2 or Th1 cytokine levels in men ([Boscolo et al., 1999](#)) and women ([Boscolo et al., 2000](#)).

Results from studies of occupationally-exposed adults also suggested that Pb exposure may be associated with decreases in Th1 cytokines and increases in Th2 cytokines; however, analyses were mostly limited to comparisons of levels among different occupational groups with different mean blood Pb levels ([Di Lorenzo et al., 2007](#); [Valentino et al., 2007](#); [Yücesoy et al., 1997a](#)) without consideration for potential confounding factors including other occupational exposures. An exception was a study of male foundry workers, pottery workers, and unexposed workers by Valentino et al. ([2007](#)). Although quantitative regression results were not provided, higher blood Pb level was associated with higher IL-10 and TNF- α with adjustment for age, BMI, smoking, and alcohol consumption. In analyses of blood Pb groups, levels of IL-2, IL-10, and IL-6 also increased from the lowest to highest blood Pb group. In contrast with most other studies, both exposed worker groups had lower IL-4 levels compared with controls. In a similar analysis, DiLorenzo et al. ([2007](#)) separated exposed workers into intermediate (9.1-29.4 $\mu\text{g/dL}$) and high (29.4-81.1 $\mu\text{g/dL}$) blood Pb level groups, with unexposed

workers comprising the low exposure group (blood Pb levels 1-11 µg/dL). Mean TNF-α levels showed a monotonic increase from the low to high blood Pb group. Levels of granulocyte colony-stimulating factor (G-CSF) did not differ between the intermediate and high blood Pb groups among the Pb recyclers; however, G-CSF levels were higher in the Pb recyclers than in the unexposed controls. Furthermore, among all subjects, blood Pb showed a strong, positive correlation with G-CSF. Yucesoy et al. ([1997a](#)) found lower serum levels of the Th1 cytokines, IL-1β and IFN-γ, in workers (mean blood Pb level of 59.4 µg/dL) compared with controls (mean blood Pb level of 4.8 µg/dL); however, levels of the Th2 cytokines, IL-2 and TNF-α levels, were similar between groups. As most occupationally-exposed cohorts represent populations highly exposed to Pb (with mean blood Pb levels >22 µg/dL), effects observed within these cohorts may not be generalizable to the population as a whole.

In summary, animal, general population, and occupational studies suggest that exposure to Pb increases the production of pro-inflammatory cytokines, skews the ratio of Th1 and Th2 cytokines to favor Th2 responses, and suppresses Th1 lymphocyte cytokine production.

4.2.6 Endocrine Disruption

4.2.6.1 Hypothalamic-Pituitary-Gonadal Axis

Evidence indicates that Pb is a potent endocrine disrupting chemical found to be associated with reproductive and developmental effects in both male and female animal models (see [Section 4.8](#)). Pb may act both at multiple points along the hypothalamic-pituitary-gonadal (HPG) axis and directly at gonadal sites. The HPG axis functions in a closely regulated manner to produce circulating sex steroids and growth factors required for normal growth and development. Long-term Pb exposure in animals (21-35 day dosing at 8 mg/kg) has been shown to alter serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, and estradiol ([Biswas and Ghosh, 2006](#); [Rubio et al., 2006](#)). Similar changes in serum HPG hormones have been observed after high-level Pb exposure in animals, resulting in blood Pb levels >20 µg/dL ([Dearth et al., 2002](#); [Ronis et al., 1998b](#); [Foster, 1992](#); [Sokol and Berman, 1991](#)). Increases in serum LH and FSH have been associated with increasing concurrent blood Pb levels in adult women from the NHANES cohort ([Krieg, 2007](#)). The change in HPG hormones likely occurs through the inhibition of LH secretion and the reduction in the expression of the steroidogenic acute regulatory protein (StAR) ([Huang and Liu, 2004](#); [Srivastava et al., 2004](#); [Huang et al., 2002](#); [Ronis et al., 1996](#)). StAR expression is the rate-limiting step

essential in maintaining gonadotropin-stimulated steroidogenesis, which results in the formation of testosterone and estradiol. Prenatal and lactational Pb exposure (via daily gavage of dams beginning 30 days prior to breeding and resulting in 3 µg/dL blood Pb in the female rat offspring at PND31) was found to decrease basal StAR synthesis, but not gonadotropin-stimulated StAR synthesis, suggesting that Pb may not directly affect ovarian responsiveness to gonadotropin stimulation ([Srivastava et al., 2004](#)). Instead, Pb may act at the hypothalamic-pituitary level to alter LH secretion, which is necessary to drive StAR production and subsequent sex hormone synthesis. Release of LH and FSH from the pituitary is controlled by gonadotropin-releasing hormone (GnRH). Pb exposure (10 µM, 90 min) in rat brain median eminence cells can block GnRH release ([Bratton et al., 1994](#)). Pb may also interfere with release of pituitary hormones through interference with cation-dependent secondary messenger systems that mediate hormone release and storage.

Endocrine disruption may also be a result of altered hormone binding to endocrine receptors. Prenatal and postnatal Pb exposure (20 ppm in drinking water) to rats was able to decrease the number of estrogen, LH, and FSH receptors found in the uterus or ovaries and receptor binding affinity ([Wiebe et al., 1988](#); [Wiebe and Barr, 1988](#)). Altered hormone binding ability may be due to the ion binding properties of Pb, resulting in changes in receptor tertiary structure that will disrupt ligand binding. In addition, Pb-induced changes in hormone levels that act as inducing agents for receptor synthesis may affect the number of hormone receptors produced.

Some of these endocrine disrupting effects of Pb have been related to the generation of ROS. Treatment with antioxidants has been able to counteract a number of the endocrine disrupting effects of Pb, including apoptosis and decreased sperm motility and production ([Salawu et al., 2009](#); [Shan et al., 2009](#); [Madhavi et al., 2007](#); [Rubio et al., 2006](#); [Wang et al., 2006a](#); [Hsu et al., 1998b](#)). Direct generation of ROS in epididymal spermatozoa was observed after Pb treatment in rats (i.p. 20 or 50 ppm, 6 weeks) ([Hsu et al., 1998a](#)). In addition, testicular lipid peroxidation has been observed in Pb-treated rats (i.p. 0.025 ppm, 15 days) ([Pandya et al., 2012](#)). Lipid peroxidation in the seminal plasma was significantly increased in a group of Pb-exposed workers with high blood Pb levels (>40 µg/dL) than in unexposed controls ([Kasperczyk et al., 2008](#)).

The liver is often associated with the HPG axis due in part to its production of insulin-like growth factor 1 (IGF-1). Children with higher concurrent blood Pb levels (>4 µg/dL) ([Huseman et al., 1992](#)) and Pb-exposed animals (blood Pb level of 14 µg/dL) ([Pine et al., 2006](#); [Dearth et al., 2002](#)) have shown a decrease in plasma IGF-1, which may be the result of decreased translation or secretion of IGF-1 ([Dearth et al., 2002](#)). IGF-1 release was also inhibited by Pb treatment in gonadal cells (46 ppm Pb exposure) ([Kolesarova et](#)

[al., 2010](#)). IGF-1 also induces LH-releasing hormone release, such that IGF-1 decrements may explain decreased LH and estradiol levels. IGF-1 production is stimulated by growth hormone (GH) secreted from the pituitary gland and could be the result of GH depletion.

A number of studies have revealed that Pb exposure affects the dynamics of growth (see [Section 4.8.1](#)). Decreased growth after Pb exposure could be the result of Pb-induced decreased GH levels ([Berry et al., 2002](#); [Camoratto et al., 1993](#); [Huseman et al., 1992](#); [Huseman et al., 1987](#)). This decrease in GH could be a result of decreased release of GH releasing hormone (GHRH) from the hypothalamus or disrupted GHRH binding to its receptor, which has been reported in vitro after Pb treatment (IC₅₀ free Pb in solution 5.2×10^{-5} μ M, 30 minutes) ([Lau et al., 1991](#)). GH secretion may also be altered from decreased testosterone resulting from Pb exposure.

4.2.6.2 Hypothalamic-Pituitary-Thyroid Axis

The evidence for the effects of Pb exposure on the hypothalamic-pituitary-thyroid (HPT) axis is mixed. Pb exposure impacts a variety of components in the thyroid hormone system. A number of occupational studies (blood Pb levels >7.3 μ g/dL) have shown that elevated blood Pb is associated with lower thyroxine (T₄) (and free T₄ levels) without alteration in triiodothyronine (T₃), suggesting that long-term Pb exposure may depress thyroid function in workers ([Dundar et al., 2006](#); [Tuppurainen et al., 1988](#); [Robins et al., 1983](#)). However, animal studies on thyroid hormones have shown mixed results. Pb-exposed cows (blood Pb levels >51 μ g/dL) were reported to have an increase in plasma T₃ and T₄ levels ([Swarup et al., 2007](#)), whereas mice and chickens manifested decreased serum T₃ concentrations after Pb exposure, which was accompanied by increased lipid peroxidation ([Chaurasia et al., 1998](#); [Chaurasia and Kar, 1997](#)). Both decreased serum T₃ and increased lipid peroxidation were restored by vitamin E treatment, suggesting the disruption of thyroid hormone homeostasis could be a result of altered membrane architecture and oxidative stress; however, no data were provided to exclude changes in Pb kinetics as the mechanism of protection ([Chaurasia and Kar, 1997](#)).

Decreased T₄ and T₃ may be the result of altered pituitary release of thyroid stimulating hormone (TSH). However, several studies have reported higher TSH levels in high-level Pb-exposed workers (blood Pb levels >39 μ g/dL) ([Lopez et al., 2000](#); [Singh et al., 2000](#); [Gustafson et al., 1989](#)), which would result in increased T₄ levels. Overall, results on the effects of Pb on the HPT axis are inconclusive.

4.2.7 Cell Death and Genotoxicity

A number of studies have attempted to characterize the genotoxicity of inorganic Pb in human populations, laboratory animals, and cell cultures. Endpoints investigated include DNA damage (single- and double-strand breaks, DNA-adduct formation), mutagenicity, clastogenicity (sister chromatid exchange, micronucleus formation, chromosomal aberrations), and epigenetic changes (changes in gene expression, DNA methylation, mitogenesis). It is important to note that numerous studies have utilized exposure to Pb chromate to investigate genotoxicity endpoints; some studies have specifically attributed the observed increases in DNA damage and clastogenicity to the chromate ion while others have not. Due to the uncertainty regarding whether observed genotoxic effects are due to chromate or Pb in studies using this form of inorganic Pb, only studies utilizing other forms of inorganic Pb (e.g., Pb nitrate, acetate, chloride, sulfate) are discussed below. Overall, evidence indicates that in vitro or in vivo exposure to various Pb compounds can increase risk of genotoxic effects, including DNA damage, clastogenicity, and mutagenicity.

4.2.7.1 DNA Damage

A number of studies in human populations have observed associations between indicators of Pb exposure and increased DNA damage, as measured as DNA strand breaks. Most of these associations have been observed in occupationally-exposed populations ([Grover et al., 2010](#); [Minozzo et al., 2010](#); [Shaik and Jamil, 2009](#); [Danadevi et al., 2003](#); [Hengstler et al., 2003](#); [Palus et al., 2003](#); [Fracasso et al., 2002](#); [de Restrepo et al., 2000](#)). Evidence overall was equivocal in regard to how blood Pb levels correlated with DNA damage: Fracasso et al. ([2002](#)) observed that DNA damage increased with increasing blood Pb levels (blood Pb levels, <25, 25-35, and >35 µg/dL), whereas Palus et al. ([2003](#)) (mean blood Pb level: 50.4 µg/dL [range: 28.2 to 65.5 µg/dL]) and Minozzo et al. ([2010](#)) (mean [SD]: 59.43 µg/dL [28.34]) observed no correlation. Hengstler et al. ([2003](#)) examined workers exposed to Pb, Cd, and cobalt (Co) and observed that neither blood (mean: 4.4 [IQR: 2.84-13.6] µg/dL) nor air Pb levels (mean: 3.0 [IQR: 1.6-50.0] µg/m³) were associated with DNA damage when examined alone, but that blood Pb influenced the occurrence of single strand DNA breaks when included in a multiple regression model along with Cd in air and blood and Co in air.

A few studies were found that investigated Pb-induced DNA damage resulting from nonoccupational exposures. Mendez-Gomez ([2008](#)) observed that children attending grade schools at close and intermediate distances to a Pb smelter had mean (range) blood Pb levels of 28.6 (11.4 to 47.5) and 19.5 (11.3 to 49.2) µg/dL, respectively, compared to

blood Pb level of 4.6 (0.1 to 8.7) $\mu\text{g/dL}$ for children living distant to the smelter. DNA damage in lymphocytes was higher in children living nearest to the smelter, compared to the children at the intermediate distance, but was not different from children living farthest away from the smelter. Multivariate analysis (which considered children urinary As levels, highest in children farthest from the smelter), revealed no statistically significant associations between DNA damage and blood Pb level. Further, DNA repair ability was also observed to be unrelated to blood Pb levels. Alternatively, Yanez et al. (2003) observed that children living close to a mining complex (mean [range] blood Pb level: 11.6 [3.0 to 19.5] $\mu\text{g/dL}$) did have higher levels of DNA damage compared to control children who lived further away from the mining facility (mean [range] blood Pb level: 8.3 [3.0 to 25.0] $\mu\text{g/dL}$).

Pb-induced DNA damage was observed in multiple animal studies. In mice exposed to Pb (blood Pb level of 0.68 $\mu\text{g/dL}$) via inhalation for up to 4 weeks, differential levels of DNA damage were observed in different organ systems, with only the lung and the liver demonstrating statistically greater DNA damage compared to the respective organ controls after acute exposure (Valverde et al., 2002). Statistically elevated levels of DNA damage were observed in the kidneys, lungs, liver, brain, nasal cavity, bone marrow, and leukocytes of mice exposed to Pb over a period of 4 weeks, although variability was high in all groups. The magnitude of the DNA damage was characterized as weak and did not increase with increasing durations of exposure. In mice given Pb nitrate (0.7 to 89.6 mg/kg) by gavage for 24, 48, or 72 hours, or 1 or 2 weeks, single strand DNA breaks in white blood cells were observed but did not increase with increasing Pb concentration (Devi et al., 2000). The three highest concentrations had responses that were similar in magnitude to each other and were actually lower than the responses to the lower concentrations tested. Xu et al. (2008) exposed mice to 10-100 mg/kg Pb acetate via gavage for four weeks and observed a concentration-dependent increase in DNA single strand breaks in white blood cells that was statistically significant at 50 and 100 mg/kg. The authors characterized the observed DNA damage as severe. Pb nitrate induced DNA damage in primary spermatozoa in rats (blood Pb levels of 19.5 and 21.9 $\mu\text{g/dL}$) over that in control rats (Nava-Hernandez et al., 2009). The level of DNA damage was not concentration dependent and was comparable in both exposure groups. Narayana and Al-Bader (2011) observed no increase in DNA damage in the livers of rats exposed to 5,000 or 10,000 ppm Pb nitrate in drinking water for 60 days. Interestingly, although the results were not statistically significant and were highly variable within exposure groups, DNA fragmentation appeared to be lower in the exposed animals.

Studies investigating Pb-induced DNA damage in human cell cultures were contradictory. Pb acetate did not induce DNA strand breaks in human HeLa cells when exposed in vitro to 500 μM Pb acetate for 20-25 hours or 100 μM for 0.5-4 hours

([Hartwig et al., 1990](#); [Snyder and Lachmann, 1989](#)). Pb nitrate, administered to lymphoma cells in vitro at 1,000-10,000 μM for 6 hours, did not result in any DNA-protein crosslinks ([Costa et al., 1996](#)). Pb acetate was observed by Woźniak and Blasiak ([2003](#)) to result in DNA single and double strand breaks in primary human lymphocytes exposed in vitro to 1-100 μM for 1 hour, although the pattern of damage was peculiar. DNA damage was greater in cells exposed to 1 or 10 μM , compared to those exposed to 100 μM . DNA-protein crosslinks were only observed in the 100 μM exposure group, suggesting that the decreased strand breaks observed in the high exposure group may be a result of increased crosslinking in this group. Pasha Shaik et al. ([2006](#)) also observed DNA damage in human lymphocytes exposed in vitro to 2,100-3,300 μM Pb nitrate for 2 hours. Although there was a concentration-dependent increase in DNA damage from 2,100-3,300 μM , no statistics were reported and no unexposed control group was included, making it difficult to interpret these results. Gastaldo et al. ([2007](#)) observed that in vitro exposure of human endothelial cells to 1-1,000 μM Pb nitrate for 24 hours resulted in a concentration-dependent increase in DNA double strand breaks.

Studies in animal cell lines collectively were equally as ambiguous as those using human cell lines. Zelikoff et al. ([1988](#)) and Roy and Rossman ([1992](#)) reported that Pb acetate (concentration not stated and 1,000 μM , respectively) did not induce single or double DNA strand breaks or DNA-protein or DNA-DNA crosslinks in CHV79 cells. However, both Xu et al. ([2006](#)) and Kermani et al. ([2008](#)) reported Pb acetate-induced DNA damage in undifferentiated PC12 cells exposed to 0.1, 1, or 10 μM for 24 hours; and in bone marrow mesenchymal stem cells exposed to 60 μM for 48 hours, respectively. Wedrychowski et al. ([1986](#)) reported that DNA-protein crosslinks were induced in a concentration-dependent manner in hepatoma cells exposed to 50-5,000 μM Pb nitrate for 4 hours. Pb acetate and Pb nitrate increased the incidence of nick translation in CHV79 cells when a bacterial DNA polymerase was added.

Pb exposure has also been shown to inhibit DNA repair mechanisms. Pb acetate did not induce single strand DNA breaks in HeLa cells exposed to 500 μM for 20-25 hours ([Hartwig et al., 1990](#)). However, exposure to both Pb acetate and UV light resulted in increased persistence of UV-induced strand breaks, compared with exposure to UV light alone. Similar effects were seen in hamster V79 cells: UV-induced mutation rates and SCE frequency was exacerbated by co-incubation with Pb acetate. Taken together, these data suggest that Pb acetate interferes with normal DNA repair mechanisms triggered by UV exposure alone. Pb nitrate was observed to affect different DNA double strand break repair pathways in human endothelial cells exposed in vitro to 100 μM for 24 hours. Exposure to Pb inhibited nonhomologous end joining repair, but increased two other repair pathways, MRE11-dependent and Rad51-related repair ([Gastaldo et al., 2007](#)).

Interestingly, in contrast to the above studies, exposure of lung carcinoma cells to 100, 300, or 500 μM Pb acetate for 24 hours resulted in an increase in nucleotide excision repair efficiency ([Li et al., 2008a](#)). Roy and Rossman ([1992](#)) observed an increase in UV-induced mutagenicity when CHV79 cells were co-exposed to 400 μM Pb acetate (a nonmutagenic concentration of Pb acetate), indicating an inhibition of DNA repair. Treatment of Chinese hamster ovary cells to 0.5-500 μM Pb acetate resulted in a concentration-dependent accumulation of apurinic/apyrimidinic site incision activity, indicating that DNA repair was diminished ([McNeill et al., 2007](#)).

4.2.7.2 Mutagenicity

Only one human study was found that investigated Pb-induced mutagenicity. Van Larebeke et al. ([2004](#)) investigated the frequency of mutations in the hypoxanthine phosphoribosyltransferase (HPRT) gene in Flemish women without occupational exposures to Pb or to a number of other heavy metals and organic contaminants. Higher blood Pb level (10th-90th percentile: 1.6 to 5.2 $\mu\text{g/dL}$) was associated with greater HPRT mutation frequency than found in the total population. Also, women with high blood Pb levels (i.e., greater than the population median, not reported) demonstrated a greater mutation frequency compared to women with lower blood Pb levels.

Pb-induced mutagenicity was investigated in a few studies using human cell cultures. Ye ([1993](#)) exposed human keratinocytes to 100 μM to 1×10^5 μM Pb acetate for 2-24 hours. This study did not measure HPRT mutations directly, but rather measured the amount of tritium (^3H) incorporated into DNA as an indicator of mutation. In the presence of 6-thioguanine, tritium incorporation was increased in exposed cells, indicating weak mutagenicity. Hwua and Yang ([1998](#)) reported that Pb acetate was not mutagenic in human foreskin fibroblasts exposed to 500-2,000 μM for 24 hours. Pb acetate remained nonmutagenic in the presence of 3-aminotriazole, a catalase inhibitor, indicating that oxidative metabolism did not play a part in potential mutagenicity of Pb. Exposure to Pb acetate alone did not induce mutagenicity in lung carcinoma cells (100-500 μM for 24 hours) or fibroblasts (300-500 μM for 24 hours) ([Li et al., 2008a](#); [Wang et al., 2008c](#)). However, pretreatment with PKC inhibitors before Pb treatment did result in statistically significant increases in mutagenicity in both cell lines.

Results from investigations into Pb-induced mutagenicity using animal cell lines were as equivocal as were the findings from human cell line studies, although the mixed findings may be reflective of specific Pb compounds used. Pb acetate was observed to be nonmutagenic (HPRT assay) in CHV79 cells exposed to 1-25 μM of the compound for 24 hours ([Hartwig et al., 1990](#)), but elicited a mutagenic response in CHV79 cells (gpt

assay) exposed to 1,700 μM for 5 days ([Roy and Rossman, 1992](#)). Pb acetate was observed to be nonmutagenic (HPRT assay) in Chinese hamster ovary cells exposed to 5 μM for 6 hours ([McNeill et al., 2007](#)). The implication of mutagenicity in the latter study is complicated by the concurrent observation of severe cytotoxicity at the same concentration. Pb nitrate was alternatively found to be nonmutagenic in CHV79 cells (gpt assay) exposed to 0.5-2,000 μM for 5 days ([Roy and Rossman, 1992](#)) but mutagenic in the same cell line (HPRT assay) exposed to 50-5,000 μM for 5 days ([Zelikoff et al., 1988](#)). However, mutagenicity was only observed at 500 μM , and was higher than that observed at higher Pb concentrations. Pb sulfate was also observed to be mutagenic in CHV79 cells (HPRT assay) exposed to 100-1,000 μM for 24 hours, but as with Pb nitrate, it was not concentration-dependent ([Zelikoff et al., 1988](#)). Pb chloride was the only Pb compound tested in animal cell lines that was consistently mutagenic: three studies from the same laboratory observed concentration-dependent mutagenicity in the gpt assay in Chinese hamster ovary cells exposed to 0.1-1 μM Pb chloride for one hour ([Ariza and Williams, 1999](#); [Ariza et al., 1998](#); [Ariza and Williams, 1996](#)).

4.2.7.3 Clastogenicity

Clastogenicity is the ability of a compound to induce chromosomal damage, and is commonly observed as sister chromatid exchange (SCE), micronuclei formation, or incidence of chromosomal aberrations (i.e., breaks or gaps in chromosomes). Pb has been shown to increase sister chromatid exchange, micronuclei formation, and chromosomal aberrations in human populations, exposed animal models, and in vitro experiments.

Sister Chromatid Exchange

An association between blood Pb levels (means: 10.48 - 86.9 $\mu\text{g/dL}$) and sister chromatid exchange (SCE) was observed in a number of occupational studies ([Wiwanitkit et al., 2008](#); [Duydu et al., 2005](#); [Palus et al., 2003](#); [Duydu et al., 2001](#); [Pinto et al., 2000](#); [Bilban, 1998](#); [Anwar and Kamal, 1988](#); [Huang et al., 1988](#)). In most studies that attempted to investigate the concentration-response relationship in workers, no association was observed between increasing blood Pb levels and the number of SCE ([Palus et al., 2003](#); [Duydu et al., 2001](#); [Pinto et al., 2000](#)). However, Huang et al. ([1988](#)) did observe increased SCE in exposed workers in the two highest blood Pb groups (52.1 and 86.9 $\mu\text{g/dL}$), with a statistically significant association observed in the 86.9 $\mu\text{g/dL}$ group. Pinto et al. ([2000](#)) did report an association with duration of exposure (range of years exposed: 1.6-40). Two studies reported no correlation between occupational exposure to Pb and number of SCE ([Rajah and Ahuja, 1996](#); [Rajah and Ahuja, 1995](#)).

Mielzynska et al. (2006) found no association between blood Pb level and SCEs in children in Poland. Children had an average blood Pb level of 7.69 µg/dL and 7.87 SCEs/cell.

Pb exposure has been observed to induce SCEs in multiple laboratory animal studies. In mice treated with up to 100 mg/kg Pb acetate i.p., Pb induced SCEs with 50 and 100 mg/kg (Fahmy, 1999). Pb nitrate, also administered i.p., induced the formation of increased SCE levels in a concentration-dependent manner (10-40 mg/kg) in the bone marrow of exposed mice (Dhir et al., 1993). Nayak et al. (1989b) treated pregnant mice with 100-200 mg/kg Pb nitrate via i.v. injection and observed an increase in the number of SCE in dams at 150 and 200 mg/kg; no increases in SCE levels were observed in the fetuses. Tapisso et al. (2009) treated rats with 21.5 mg/kg Pb acetate (1/10th the LD₅₀) via i.p. injection on alternating days for 11 or 21 days, for a total of 5 or 10 treatments. Induction of SCE in the bone marrow of exposed rats was increased over controls in a statistically significant duration-dependent manner. It is important to note that all of these studies utilized an injection route of exposure that may not be relevant to routes of exposure in the human population (e.g., air, drinking water exposure).

Few studies were found that investigated SCE formation due to Pb exposure in human cell lines. Statistically significant, concentration-dependent increases in SCE were observed in human lymphocytes obtained from a single donor when incubated with 1, 5, 10, or 50 µM Pb nitrate (Üstundag and Duydu, 2007). Melatonin and N-acetylcysteine were reported to ameliorate these effects, indicating Pb may induce increases in SCE levels through increased oxidative stress. Pb chloride was also observed to increase SCE levels in human lymphocytes exposed to 3 or 5 ppm (Turkez et al., 2011).

Evidence from studies investigating SCE in rodent cells was more equivocal than that in human cells. Pb sulfate, acetate, and nitrate were found not to induce SCE in CHV79 cells (Hartwig et al., 1990; Zelikoff et al., 1988). Both of these studies only examined 25-30 cells per concentration, reducing their power to detect Pb-induced increases in SCE levels. Cai and Arenaz (1998), on the other hand, used 100 cells per treatment and observed that exposure to 0.05-1 µM Pb nitrate for 3-12 hours resulted in a weak, concentration-dependent increase in SCE levels in Chinese hamster ovary cells. Lin et al. (1994) also observed a concentration-dependent increase in SCE levels in Chinese hamster cells exposed to 3-30 µM Pb nitrate for 2 hours.

Micronucleus Formation

Pb-induced micronucleus formation was observed in numerous occupational studies ([Grover et al., 2010](#); [Khan et al., 2010b](#); [Minozzo et al., 2010](#); [Shaik and Jamil, 2009](#); [Minozzo et al., 2004](#); [Palus et al., 2003](#); [Vaglenov et al., 2001](#); [Pinto et al., 2000](#); [Bilban, 1998](#); [Vaglenov et al., 1998](#)). Pinto et al. (2000) observed increased micronuclei in exposed workers with an average blood Pb level of 10.48 µg/dL compared with unexposed controls. In studies investigating the correlation between blood Pb levels and micronucleus formation, no association was observed ([Minozzo et al., 2010](#); [Minozzo et al., 2004](#); [Palus et al., 2003](#); [Pinto et al., 2000](#)), although Pinto et al. (2000), Grover et al. (2010), and Minozzo et al. (2010) did report an association between micronuclei formation and duration of exposure. Mielzynska et al. (2006) investigated micronucleus formation in a nonworker population and reported a statistically significant positive correlation between blood Pb levels and micronuclei frequency in children in Poland. Children, with an average blood Pb level of 7.69 µg/dL, were observed to have 4.44 micronucleated cells per 1,000 cells analyzed. Children with blood Pb levels greater than 10 µg/dL had significantly more micronucleated cells than did children with blood Pb levels less than 10 µg/dL.

Micronucleus formation in response to Pb exposure has been observed in rodent animal studies. Celik et al. (2005) observed that exposure of female rats to Pb acetate (140, 250, or 500 mg/kg once per week for 10 weeks) resulted in statistically significant increases in numbers of micronucleated polychromatic erythrocytes (PCEs) compared to controls. Similarly, Alghazal et al. (2008b) exposed rats to Pb acetate (100 ppm daily for 125 days) and observed statistically significant increases in micronucleated PCEs in both sexes. Tapisso et al. (2009) treated rats with Pb acetate (21.5 mg/kg; 1/10th the LD₅₀) via i.p. injection on alternating days for 11 or 21 days, for a total of 5 or 10 exposures. Formation of micronuclei in the bone marrow of exposed rats was increased over formation in controls in a significant duration-dependent manner. Two further studies investigated formation of micronuclei in the bone marrow of exposed mice: Roy et al. (1992) treated mice with Pb nitrate (10 or 20 mg/kg, i.p.) and observed a concentration-dependent increase in micronuclei, whereas Jagetia and Aruna (1998) observed an increase in micronuclei in mice treated with Pb nitrate (0.625-80 mg/kg, i.p.), though the increase was not concentration-dependent. Mice exposed to Pb acetate (0.1 µg/L via drinking water, a more environmentally relevant route of exposure, for 90 days) had statistically significant increases in micronucleated PCEs ([Marques et al., 2006](#)).

A few studies were found that reported increased micronucleus formation in human cell lines treated with Pb. Concentration-dependent micronucleus formation was observed in human lymphocytes when exposed in vitro to either 1, 5, 10, or 50 µM Pb nitrate or 3 or 5 ppm Pb chloride ([Turkez et al., 2011](#); [Üstundag and Duydu, 2007](#)). Gastaldo et al.

(2007) also observed a concentration-dependent increase in micronuclei in human endothelial cells exposed in vitro to 1-1,000 μM Pb nitrate for 24 hours. Animal cell culture studies investigating micronuclei formation produced contrasting results. One study observed that micronuclei were not induced in Chinese hamster cells exposed to 3-30 μM Pb nitrate for 2 hours (Lin et al., 1994), whereas the other observed that Pb acetate induced a concentration-dependent increase in Chinese hamster cells when administered at 0.03-10 μM for 18 hours (Bonacker et al., 2005).

Chromosomal Aberrations

Chromosomal aberrations (e.g., chromosome breaks, nucleoplasmic bridges, di- and acentric chromosomes, and rings) were examined in a number of occupational studies (Grover et al., 2010; Shaik and Jamil, 2009; Pinto et al., 2000; Bilban, 1998; De et al., 1995; Huang et al., 1988). No correlation was observed between increasing blood Pb level and the number of chromosomal aberrations, although an association was observed between duration of exposure and chromosomal damage (Grover et al., 2010; Pinto et al., 2000). Other studies reported no association between occupational exposure to Pb and chromosomal aberrations (Anwar and Kamal, 1988; Andreae, 1983). Smejkalova (1990) observed greater chromosomal damage and aberrations in children living in a heavily Pb-contaminated area of Czechoslovakia compared with children living in an area with less contamination, although the difference between the two areas was not statistically significant. Blood Pb levels were comparable between children living in the Pb-contaminated area and children living in the less contaminated area (low 30s versus high 20s $\mu\text{g/dL}$, respectively), indicating there may not be enough of a dose contrast to detect a significant difference in aberration rates.

The majority of animal studies investigating Pb-induced genotoxicity focused on the capacity of Pb to produce chromosomal damage. Fahmy (1999) treated mice with Pb acetate (25-400 mg/kg i.p.), either as a single dose or repeatedly for 3, 5, or 7 days. Chromosomal damage was observed to increase in bone marrow cells (100-400 mg/kg) and spermatocytes (50-400 mg/kg) in a concentration-dependent manner after both dosing regimens. Pb nitrate was also observed to produce concentration-dependent chromosomal damage in mice treated i.p. to a single dosage of 5, 10, or 20 mg/kg (Dhir et al., 1992b). In a similar experiment, Dhir et al. (1990) treated mice with Pb nitrate (10, 20, or 40 mg/kg) and saw an increase in chromosomal aberrations, although there was no concentration-dependent response as the response was similar in all concentrations tested. Nayak et al. (1989b) treated pregnant mice with Pb nitrate (100, 150, or 200 mg/kg via i.v. injection) and observed no chromosomal gaps or breaks in dams or fetuses but did report some karyotypic chromosomal damage and weak aneuploidy at the low dose. In a similar experiment, low levels of chromosomal aberrations were observed in dams and

fetuses injected with Pb nitrate (12.5, 50, or 75 mg/kg), but there was no concentration-dependent response reported and few cells were analyzed ([Nayak et al., 1989a](#)). In rats given Pb acetate (2.5 mg/100 g body weight, i.p. daily for 5-15 days or 10-20 mg/100 g once and analyzed after 15 days), Pb-induced chromosomal aberrations were observed ([Chakraborty et al., 1987](#)). The above studies all are limited by the use of a route of exposure that may not be relevant to human environmental exposures. However, studies utilizing oral exposures also observed increases in chromosomal damage. Aboul-Ela ([2002](#)) exposed mice to Pb acetate (200 or 400 mg/kg by gavage for 5 days) and reported that chromosomal damage was present in the bone marrow cells and spermatocytes of animals exposed to both concentrations. Dhir et al. ([1992a](#)) also observed a concentration-dependent increase in chromosomal damage in mice exposed via gavage, albeit at much lower concentrations: either 5 or 10 mg/kg. Nehez et al. ([2000](#)) observed a Pb-induced increase in aneuploidy and percent of cells with damage after exposure to 10 mg/kg administered by gavage 5 days a week for 4 weeks. In the only study that investigated dietary exposure, El-Ashmawy et al. ([2006](#)) exposed mice to 5,000 ppm Pb acetate in feed, and observed an increase in abnormal cells and frequency of chromosomal damage.

In the few studies that investigated the capacity of Pb to induce chromosomal damage in human cell lines, Pb exposure did not induce chromosomal damage. Wise et al. ([2005](#); [2004](#)) observed that Pb glutamate was not mutagenic in human lung cells exposed in vitro to 250-2,000 μ M for 24 hours. Pasha Shaik et al. ([2006](#)) observed that Pb nitrate did not increase chromosomal aberrations in primary lymphocytes (obtained from healthy volunteers) when incubated with 1,200 or 2,000 μ M for 2 hours. Studies utilizing animal cell lines generally supported the finding of no Pb-induced chromosomal damage in human cell lines. Pb nitrate was found to induce no chromosomal damage in Chinese hamster ovary cells exposed to 500-2,000 μ M for 24 hours ([Wise et al., 1994](#)), 3-30 μ M for 2 hours ([Lin et al., 1994](#)), or 0.05-1 μ M for 3-12 hours ([Cai and Arenaz, 1998](#)). Wise et al. ([1994](#)) did observe increased chromosomal damage in Chinese hamster ovary cells exposed to 1,000 μ M Pb glutamate for 24 hours but did not see any damage in cells exposed to higher concentrations (up to 2,000 μ M).

4.2.7.4 Epigenetic Effects

Epigenetic effects are heritable changes in gene expression resulting without changes in the underlying DNA sequence. A prime example of an epigenetic effect is the abnormal methylation of DNA, which could lead to altered gene expression and cell proliferation and differentiation. Possible indications of Pb-induced epigenetic changes include alterations in methylation patterns in exposed rats, and alterations in mitogenesis and cell proliferation in exposed humans and animals, as well as human and animal cell cultures.

DNA Methylation

A single i.v. injection of Pb nitrate (75 µmol/kg) resulted in global hypomethylation of hepatic DNA in rats ([Kanduc et al., 1991](#)). The observed hypomethylation in the liver was associated with an increase in cell proliferation. A few additional studies in humans observed that higher bone Pb levels were associated with lower global DNA methylation patterns in adults and cord blood of newborns ([Wright et al., 2010](#); [Pilsner et al., 2009](#)). Hypomethylation specifically is associated with increased gene expression. Changes in DNA methylation patterns could potentially lead to dysregulation of gene expression and altered tissue differentiation.

Mitogenesis

Conflicting results have been reported regarding Pb-induced effects on mitogenesis, with both increased and decreased cell growth and mitogenesis. A discernible pattern of effects is difficult to detect when analyzing effects across human, in vivo animal, and in vitro studies. Only a few studies have investigated the epigenetic effects of Pb exposure in human populations indirectly by examining mitogenesis or the induction of cell proliferation, which can be a consequence of epigenetic changes. These studies ([Minozzo et al., 2010](#); [Minozzo et al., 2004](#); [Rajah and Ahuja, 1995](#)) reported reduced mitogenesis in two groups of Pb-exposed workers compared with unexposed controls (mean blood Pb levels: 35.4 µg/dL, 59.4 µg/dL, and not reported, respectively). The observation of decreased cell division in exposed workers may indicate that cells suffered DNA damage and died during division, or that division was delayed to allow for DNA repair to occur. It is also possible that Pb exerts an aneugenic effect and arrests the cell cycle.

Many studies have investigated the ability of Pb to induce mitogenesis in animal models, and have consistently shown that Pb nitrate can stimulate DNA synthesis and cell proliferation in the liver of animals treated with 100 µM Pb per kg body weight, via i.v. injection ([Nakajima et al., 1995](#); [Coni et al., 1992](#); [Ledda-Columbano et al., 1992](#); [Columbano et al., 1990](#); [Columbano et al., 1987](#)). Shinozuka et al. ([1996](#)) observed that

Pb-induced hepatocellular proliferation was similar in magnitude to that induced by TNF- α at 100 μ M/kg; and Pb was observed to induce TNF- α in glial and nerve cells in mice (and NF- κ B, TNF- α , and iNOS in rat liver cells) from mice treated with Pb at 12.5 mg/kg and 100 μ mol/kg, respectively ([Cheng et al., 2002](#); [Menegazzi et al., 1997](#)). The only study that examined Pb exposure via inhalation (Pb acetate, 10,000 μ M for 4 weeks) found increased cellular proliferation in murine lungs ([Fortoul et al., 2005](#)).

Extensive research has been conducted investigating the potential effects of Pb on mitogenesis in human and animal cell cultures. In human cell cultures, Pb acetate inhibited cell growth in hepatoma cells (0.1-100 μ M for 2-6 days) ([Heiman and Tonner, 1995](#)) and primary oligodendrocyte progenitor cells (1 μ M for 24 hours) ([Deng and Poretz, 2002](#)) but had no observable effects on growth in glioma cells (0.01-10 μ M for 12-72 hours) ([Liu et al., 2000](#)). Pb glutamate had no effect on cell growth in human lung cells in vitro, but did increase the mitotic index (250-1,000 μ M exposure for 24 hours) ([Wise et al., 2005](#)). The increase in the mitotic index was attributed to an arrest of the cell cycle at M-phase, and was not attributed to an actual increase of cell growth and proliferation. Gastaldo et al. ([2007](#)) also reported S and G2 cell cycle arrests in human endothelial cells following exposure to 100 μ M Pb nitrate for 24 hours. Conflicting results with regard to DNA synthesis were reported, with a concentration-dependent inhibition of DNA synthesis reported in hepatoma cells (1-100 μ M for 72 hours) ([Heiman and Tonner, 1995](#)), but an induction of synthesis observed in astrocytoma cells (1-50 μ M for 24 hours) ([Lu et al., 2002](#)).

In rat fibroblasts and epithelial cells, Pb acetate, Pb chloride, Pb oxide, and Pb sulfate were all observed to inhibit cell growth (10-1,000 μ M for 1-7 days and 0.078-320 μ M for 48 hours, respectively) ([Iavicoli et al., 2001](#); [Apostoli et al., 2000](#)). Iavicoli et al. ([2001](#)) observed that in addition to inhibiting cell growth in rat fibroblasts, Pb acetate caused G2/M and S-phase arrest. Pb acetate decreased cell proliferation in mouse bone marrow mesenchymal stem cells when administered at 20-100 μ M for 48 hours ([Kermani et al., 2008](#)). Pb nitrate was alternatively reported to increase ([Lin et al., 1994](#)) and decrease ([Cai and Arenaz, 1998](#)) the mitotic index in Chinese hamster ovary cells exposed to 1 μ M Pb nitrate. Lin et al. ([1994](#)) did not consider cell cycle arrest when measuring the mitotic index and did not observe a decrease at higher concentrations; in fact, the highest concentration tested, 30 μ M, had a mitotic index equal to that in the untreated control cells.

4.2.7.5 Gene Expression

A few animal studies have investigated the ability of Pb exposure to alter gene expression in regard to phase I and II metabolizing enzymes. Suzuki et al. (1996) treated rats with Pb acetate or Pb nitrate (100 µg/kg via i.p. injection) and observed an induction of GST-P with both Pb compounds. The induction of GST-P by Pb was observed to occur on the transcriptional level and to be dependent on the direct activation of the cis-element GPEI enhancer. Degawa et al. (1993) reported that Pb nitrate (20, 50, or 100 µmol/kg, via i.v.) selectively inhibited CYP1A2 levels. Pb was shown to inhibit CYP1A2 not by direct enzyme inhibition, but rather to decrease the amount of CYP1A2 mRNA. In contrast, Korashy and El Kadi (2004) observed that exposure of murine hepatoma cells to Pb nitrate (10-100 µM for 24 hours) increased the amount of CYP1A1 mRNA while not influencing the activity of the enzyme. NAD(P)H:quinone oxidoreductase and GST Ya activities and mRNA levels were increased after exposure to Pb. Incubation of primary human bronchial epithelial cells with Pb acetate (500 µg/L for 72 hours) resulted in the up-regulation of multiple genes associated with cytochrome P450 activity, glutathione metabolism, the pentose phosphate pathway, and amino acid metabolism (Glahn et al., 2008).

Additional animal studies provide further evidence that exposure to Pb compounds can perturb gene expression. Zawia and Harry (1995) investigated whether the observed Pb-induced disruption of myelin formation in rat pups exposed postnatally was due to altered gene expression. In pups exposed to 2,000 ppm Pb acetate via lactation from PND1-PND20, the expression of proteolipid protein, a major structural constituent of myelin, was elevated (statistically significant) at PND20, compared to controls. The expression of another structural element of myelin (myelin basic protein) was similarly elevated in exposed animals, although not significantly so. The expression of both genes returned to control levels 5 days following the termination of exposure. These data suggest that altered gene expression in structural myelin proteins due to Pb exposure may be responsible for observed alterations in abnormal conduction of nerve impulses. Long et al. (2011) investigated the Pb-induced increase in ABCC5, an ATP-binding cassette transporter, in embryonic and adult zebrafish. In the initial in vitro portion of the study, exposure of zebrafish fibroblasts to 20 µM Pb nitrate for 24 hours significantly increased the induction of ABCC5 mRNA 2.68-fold over controls. Similar levels of induction were observed when embryonic zebrafish were exposed to 5 µM for 24 to 96 hours; specifically, induction of ABCC5 was seen in the livers of developing embryos. In adult fish, induction of ABCC5 was observed in the brains, intestines, and kidneys of exposed fish, but a decrease was found in their livers. Induction of ABCC5 in adult fish was observed to attenuate the toxicity of Cd (but not Hg or As); however, in developing embryos, the attenuation of Pb-induced toxicity was not investigated. These findings

indicate that increased expression of ABCC5 due to heavy metal exposure may play a part in cellular defense mechanisms.

4.2.7.6 Apoptosis

Occupational exposure to Pb and induction of apoptosis in various cell types was investigated in a few studies. The study that directly measured apoptosis reported that exposure to Pb increased apoptosis of lymphocytes compared to nonexposed controls ([Minozzo et al., 2010](#)), whereas the others reported that two early indicators of apoptosis, karyorrhexis and karyolysis, were elevated in occupationally exposed workers ([Grover et al., 2010](#); [Khan et al., 2010b](#)). Pb nitrate was also observed to induce apoptosis in the liver of exposed animals ([Columbano et al., 1996](#); [Nakajima et al., 1995](#)). Apoptosis was observed in rat fibroblasts exposed in vitro to Pb acetate and rat alveolar macrophages exposed to Pb nitrate ([Iavicoli et al., 2001](#); [Shabani and Rabbani, 2000](#)). Observation of Pb-induced apoptosis may represent the dysregulation of genetically-controlled cell processes and tissue homeostasis.

4.2.8 Summary

The diverse health effects of Pb are mediated through multiple, interconnected modes of action. Each of the modes of action discussed here has the potential to contribute to the development of a number of Pb-induced health effects ([Table 4-2](#)). While this section draws from earlier literature as well as newer lines of evidence, the inclusion of recent evidence does not qualitatively change the previous conclusions regarding individual modes of action. Rather, the more recent evidence agrees with, and thus strengthens these conclusions. Evidence for the majority of these modes of action is observed with concurrent blood Pb levels in humans ranging between 2 and 17 µg/dL, with supporting evidence from animal and in vitro assays. As many of these studies examined adults, with likely higher past than current Pb exposures, uncertainty exists as to the Pb exposure level, duration, frequency, and timing associated with these modes of action. The blood Pb levels or in vitro concentrations presented in [Table 4-2](#) reflect the current evidence for these modes of action and are not intended to convey conclusions regarding specific thresholds. Also, the data presented in this table do not inform the exposure frequency and duration required to elicit a particular MOA.

Table 4-2 MOAs, their related health effects, and information on concentrations eliciting the MOAs.

Mode of Action [Related Health Effects (ISA Section)]	Concentrations or Doses (Conditions) ^a	
	Blood Pb	Dose
Altered Ion Status [All Health Effects of Pb, Chapter 4]	3.5 µg/dL (Mean in cord blood; association with cord blood Ca ²⁺ ATPase pump activity) Huel et al. (2008)	0.00005 µM free Pb ²⁺ (In vitro; 30 minutes; calmodulin activation assay) Kern et al. (2000)
Protein Binding [Renal (4.5), Hematological Effects (4.7)]	17.0 µg/dL (Concurrent mean in adult workers with wildtype metallothionein expression; increased BP susceptibility) Chen et al. (2010a)	50 µM Pb glutamate (In vitro; 24 hours; increased nuclear protein in neurological cell) Klann and Shelton (1989)
Oxidative Stress [All Health Effects of Pb, Chapter 4]	5.4 µg/dL (Concurrent mean in adult male workers; decreased CAT activity in blood) Conterato et al. (2013)	0.1 µM Pb acetate (In vitro; 48 hours; decreased cellular GSH in neuroblastoma cells) Chetty et al. (2005)
Inflammation [Nervous System (4.3), Cardiovascular (4.4), Renal (4.5), Immune (4.6), Respiratory (4.9.6), Hepatic (4.9.1)]	Among males with concurrent blood Pb ≥ 2.5 µg/dL (Increased serum TNF-α and blood WBC count) Kim et al. (2007)	0.01 µM Pb acetate (In vitro; 48 hours; increased cellular PGE ₂ in neuroblastoma cells) Chetty et al. (2005)
Endocrine Disruption [Reproductive and Developmental Effects (4.8), Endocrine System (4.9.3), Bone and Teeth (4.9.4)]	1.7 µg/dL (Lowest level at which a relationship could be detected in adult women with both ovaries removed; increased serum FSH) Krieg (2007)	10 µM Pb nitrate (In vitro; 30 minutes; displaced GHRH binding to rat pituitary receptors) Lau et al. (1991)
Cell Death/Genotoxicity [Cancer (4.10), Reproductive and Developmental Effects (4.8), Bone and Teeth (4.9.4)]	3.3 µg/dL (Concurrent median in adult women; increased rate of HPRT mutation frequency) Van Larebeke et al. (2004)	0.03 µM Pb acetate (In vitro; 18 hours; increased formation of micronuclei) Bonacker et al. (2005)

^aThis table provides examples of studies that report effects with low Pb dosages or concentrations; they are not the full body of evidence used to characterize the weight of the evidence. In addition, the levels cited are reflective of the data and methods available and do not imply that these modes of action are not acting at lower Pb exposure or blood Pb levels or that these doses represent the threshold of the effect. Additionally, the blood concentrations and doses (indicating Pb concentrations from in vitro systems) refer to the concentrations and doses at which these modes of action were observed. While the individual modes of action are related back to specific health effects sections (e.g., Nervous System, Cardiovascular), the concentrations and doses given should not be interpreted as levels at which those specific health effects occur. Also, the data presented in this table do not inform the exposure frequency and duration required to elicit a particular MOA.

The alteration of cellular ion status (including disruption of Ca²⁺ homeostasis, altered ion transport mechanisms, and perturbed protein function through displacement of metal cofactors) appears to be the major unifying mode of action underlying all subsequent modes of action (Figure 4-1). Pb is well characterized to interfere with endogenous Ca²⁺

homeostasis (necessary as a cell signal carrier mediating normal cellular functions). $[\text{Ca}^{2+}]_i$ has been shown to increase after Pb exposure in a number of cell types including bone, erythrocytes, brain cells, and white blood cells, due to the increased flux of extracellular Ca^{2+} into the cell. This disruption of ion transport is due in part to the alteration of the activity of transport channels and proteins, such as Na^+/K^+ ATPase and voltage-gated Ca^{2+} channels. Pb can interfere with these proteins through direct competition between Pb and the native metals present in the protein metal binding domain or through disruption of proteins important in Ca^{2+} -dependent cell signaling, such as PKC or calmodulin.

Disruption of ion transport not only leads to altered Ca^{2+} homeostasis, but it can also result in perturbed neurotransmitter function. Pb has been shown to displace metal ions, (such as Zn^{2+} , Mg^{2+} , and Ca^{2+}) from proteins due to the flexible coordination number of Pb and multiple ligand binding ability, leading to abnormal conformational changes in proteins and altered protein function. Evidence for this metal ion displacement and protein perturbation has been shown at 10^{-6} μM concentrations of Pb. Additional effects of altered cellular ion status are the inhibition of heme synthesis and decreased cellular energy production due to perturbation of mitochondrial function.

Although Pb can bind to proteins within cells through interactions with side group moieties, thus potentially disrupting cellular function, protein binding of Pb may represent a mechanism by which cells protect themselves against the toxic effects of Pb. Intranuclear and intracytosolic inclusion body formation has been observed in the kidney, liver, lung, and brain following Pb exposure. A number of unique Pb binding proteins have been detected, constituting the observed inclusion bodies. The major Pb binding protein in blood is ALAD with carriers of the ALAD-2 allele potentially exhibiting higher Pb binding affinity. Additionally, metallothionein is an important protein in the formation of inclusion bodies and mitigation of the toxic effects of Pb.

A second major mode of action of Pb is its role in the development of oxidative stress, due in many instances to the antagonism of normal metal ion functions. The origin of oxidative stress produced after Pb exposure is likely a multipathway process, resulting from oxidation of δ -ALA, NAD(P)H oxidase activation, membrane and lipid peroxidation, and antioxidant enzyme depletion. Through the inhibition of δ -ALAD (due to displacement of Zn by Pb), accumulated δ -ALA goes through an auto-oxidation process to produce ROS. Additionally, Pb can induce the production of ROS through the activation of NAD(P)H oxidase. Pb-induced ROS can interact with membrane lipids to cause a membrane and lipid peroxidation cascade. Enhanced lipid peroxidation can also result from Pb potentiation of Fe^{2+} initiated lipid peroxidation and alteration of membrane composition after Pb exposure. Increased Pb-induced ROS can also sequester and

inactivate biologically active NO, leading to the increased production of the toxic product nitrotyrosine, increased compensatory NOS, and decreased sGC protein. Pb-induced oxidative stress not only can result from increased ROS production but also through the alteration and reduction in activity of the antioxidant defense enzymes. The biological actions of a number of these enzymes are antagonized due to the displacement of the protein functional metal ions by Pb.

In a number of organ systems, Pb-induced oxidative stress is accompanied by misregulated inflammation. Pb exposure can modulate inflammatory cell function, production of pro-inflammatory cytokines and metabolites, inflammatory chemical messengers, and pro-inflammatory signaling cascades. Cytokine production is skewed toward the production of pro-inflammatory cytokines like TNF- α and IL-6 as well as toward the promotion of a Th2 response and suppression of a Th1 response accompanied by a corresponding shift in the production of related cytokines.

Evidence indicates that Pb is a potent endocrine disrupting chemical. Pb can disrupt the HPG axis evidenced by altered serum hormone levels, such as FSH, LH, testosterone, and estradiol. Pb can interact with the hypothalamic-pituitary level hormone control causing a decrease in pituitary hormones, alteration of growth dynamics due to decreased IGF-1, inhibition of LH secretion, and reduction in StAR protein. Pb has also been shown to alter hormone receptor binding likely due to interference of metal cations with secondary messenger systems and receptor ligand binding and through generation of ROS. Pb also may disrupt the HPT axis by alteration of a number of thyroid hormones, possibly due to oxidative stress. However, the results of these studies investigating HPT are mixed.

The association of Pb with increased genotoxicity and cell death has been investigated in humans, animals, and cell models. Occupational Pb exposure in humans has been associated with increased DNA damage; however, lower blood Pb and exposure levels also have been associated with these effects in experimental animals and cells. While not entirely consistent, a number of studies reported decreased repair processes following Pb exposure. There is evidence of mutagenesis and clastogenicity in highly-exposed humans; however, weak evidence has been shown in animals and cell based systems. Human occupational studies provide limited evidence for micronucleus formation (blood Pb levels >10 $\mu\text{g/dL}$) and are supported by Pb-induced effects in both animal and cell studies at higher exposure levels. Animal studies have also provided evidence for Pb-induced chromosomal aberrations. The observed increases in clastogenicity may be the result of increased oxidative damage to DNA due to Pb exposure, as co-exposures with antioxidants ameliorate the observed toxicities. Limited evidence of epigenetic effects is available, including abnormal DNA methylation, mitogenesis, and gene

expression. Pb may alter gene expression by displacing Zn from multiple transcriptional factors, thus perturbing their normal cellular activities. Consistently positive results have provided evidence of increased apoptosis following Pb exposure.

Similar to Pb, other polyvalent metal ions (e.g., Cd, Cr, Be, Ba, Se, Sr, As, Al, Cu) have demonstrated molecular mimicry and displacement of biological cations ([Garza et al., 2006](#)). In this manner, these metal ions share with Pb a common central mode of action of disruption of ion status. Specifically, these metals have been shown to disrupt cellular processes as diverse as Ca^{2+} homeostasis, cell signaling, neurotransmitter release, cation membrane channel function, protein-DNA binding, and cellular membrane structure ([Pentyala et al., 2010](#); [Huang et al., 2004](#); [Atchison, 2003](#); [Jehan and Motlag, 1995](#); [Richardt et al., 1986](#); [Cooper and Manalis, 1984](#); [Habermann et al., 1983](#)). Additionally, presumably through their shared central mode of action, some of these metal ions also display corresponding downstream modes of actions such as oxidative stress, apoptosis, and genotoxicity ([Jomova and Valko, 2011](#); [Jomova et al., 2011](#); [Matović et al., 2011](#); [Agarwal et al., 2009](#); [Méndez-Gómez et al., 2008](#); [Rana, 2008](#); [Hengstler et al., 2003](#)).

Overall, Pb-induced health effects can occur through a number of interconnected modes of action that generally originate with the alteration of ion status.

4.3 Nervous System Effects

4.3.1 Introduction

The 2006 Pb AQCD concluded that the “overall weight of the available evidence provides clear substantiation of neurocognitive decrements being associated in young children with blood-Pb concentrations...” ([U.S. EPA, 2006b](#)). This conclusion was based on evidence from several prospective and cross-sectional epidemiologic studies conducted in diverse populations with adjustment for potential confounding by socioeconomic status (SES) as well as parental intelligence, education, and caregiving quality and stimulation. The association between blood Pb levels and cognitive function decrements was further supported by an international pooled analysis of children, ages 4.8 to 10 years, participating in seven prospective studies (Boston, MA; Cincinnati, OH; Rochester, NY; Cleveland, OH; Mexico City, Mexico; Port Pirie, Australia; and Kosovo, Yugoslavia) ([Lanphear et al., 2005](#)). Across all previously evaluated studies, associations between blood Pb levels and decrements in full-scale intelligence quotient (FSIQ), infant mental development, memory, learning, and executive function were found in children ages 2 to 17 years with population mean blood Pb levels (measured at various lifestages and time periods) 5-10 $\mu\text{g}/\text{dL}$; however, several results indicated associations in groups

of children (ages 2-10 years) with mean blood Pb levels in the lower range of 3-5 µg/dL ([Bellinger, 2008](#); [Canfield, 2008](#); [Hornung, 2008](#); [Téllez-Rojo, 2008](#)). Based on fewer available studies, the 2006 Pb AQCD described evidence from prospective and cross-sectional epidemiologic studies for associations of childhood blood Pb levels with attention decrements in children ages 6-17 years and measures of conduct disorders in children ages 4-17 years and young adults ages 18-21 years ([U.S. EPA, 2006b](#)).

Biological plausibility for epidemiologic evidence in children was provided by similarly consistent toxicological findings for Pb-induced impairments in learning and behavior in rodents and monkeys ([U.S. EPA, 2006b](#)). Pb exposure was not found consistently to affect the memory of animals. In animals, learning impairments were demonstrated largely as poorer performance in maze tests, shorter interresponse times on schedule controlled behavior tasks, and response perseveration errors in discrimination reversal tests. Some results from these tests also indicated Pb-induced increases in impulsivity. Pb-induced impulsivity in animals also was demonstrated as increased response rates on the Fixed Ratio (FR)/waiting for reward test. These effects on learning and behavioral impairments in animals were found predominately with Pb exposures that resulted in blood Pb levels 30-50 µg/dL; however, some studies observed these impairments in rodents (pre- and post-natal Pb exposure) and monkeys (postnatal Pb exposure) with blood Pb levels 14-25 µg/dL ([Kuhlmann et al., 1997](#); [Altmann et al., 1993](#); [Rice and Karpinski, 1988](#); [Gilbert and Rice, 1987](#)). Toxicological studies provided additional biological plausibility for Pb-induced impairments in learning and behavior by demonstrating effects on endpoints related to modes of action. Evidence for Pb affecting neuronal development and function at the cellular and subcellular level (e.g., blood brain barrier integrity, synaptic architecture during development, neurite outgrowth, glial growth, neurotransmitter release, oxidative stress), provided biological plausibility for associations observed in children between blood Pb levels and decrements in multiple functional domains such as cognitive function, memory, motor function, mood, and behavioral problems. Additional biological plausibility was provided by associations observed of childhood blood Pb levels with changes indicative of neuronal damage and altered brain physiology in small groups of children ([Meng et al., 2005](#); [Trope et al., 2001](#)) and young adults ([Yuan et al., 2006](#); [Cecil et al., 2005](#)) assessed using magnetic resonance imaging techniques.

A common finding across several different populations of children was a supralinear concentration-response relationship between blood Pb level and cognitive function decrements, i.e., a larger decrement in cognitive function per unit increase in blood Pb level in children in the lower range of the study population blood Pb level distribution. Particularly compelling evidence was provided by studies that examined prenatal or early childhood blood Pb levels or considered peak blood Pb levels in school-aged children

([Schnaas et al., 2006](#); [Bellinger and Needleman, 2003](#); [Canfield et al., 2003a](#)) or examined concurrent blood Pb levels in young children age 2 years. ([Téllez-Rojo et al., 2006](#)). Most of these epidemiologic studies used a cut-point of 10 µg/dL to define lower and higher blood Pb levels. Consistent with these individual study findings, results from the pooled analyses of seven cohorts indicated that a nonlinear relationship fit the data better than a linear relationship ([Lanphear et al., 2005](#); [Rothenberg and Rothenberg, 2005](#)). Explanations for the supralinear concentration-response were not well characterized.

Another area of focus was the comparison of various lifestages and time periods of Pb exposure with respect to increased risk of neurodevelopmental decrements. Animal studies clearly demonstrated that gestational Pb exposure with or without additional early postnatal exposure resulted in neurodevelopmental impairments. However, gestational Pb exposure was not necessary as neurodevelopmental effects in animals also occurred with postnatal juvenile-only and lifetime Pb exposures. Epidemiologic studies observed decrements in cognitive function in children ages 2 to 17 years in association with prenatal, peak childhood, cumulative childhood, and concurrent blood Pb levels. Although examined in few studies, tooth or bone Pb levels were associated with cognitive function decrements and behavioral problems in children and adolescents ([Wasserman et al., 2003](#); [Bellinger et al., 1994b](#); [Fergusson et al., 1993](#); [Needleman et al., 1979](#)), potentially pointing to an effect of cumulative or early childhood Pb exposure. Among studies of children ages 3-10 years that examined blood Pb levels measured at multiple lifestages and time periods, several found that concurrent blood Pb was associated with a similar magnitude or larger decrement in FSIQ than blood Pb levels measured earlier in childhood or averaged over multiple years ([Lanphear et al., 2005](#); [Canfield et al., 2003a](#); [Wasserman et al., 1994](#); [Dietrich et al., 1993b](#)). A common limitation of studies of Pb and neurodevelopment in children is the high correlation among blood Pb levels at different ages, making it difficult to identify an individual critical lifestage or duration of Pb exposure associated with risk of neurodevelopmental decrements ([Lanphear et al., 2005](#)).

Some evidence indicated the persistence of neurodevelopmental effects of Pb exposure, by associations of biomarkers of earlier childhood Pb exposure (e.g., deciduous tooth Pb, blood Pb prenatally or at age 2 or 6 years) with cognitive function decrements and behavioral problems later in childhood or as young adults ([Ris et al., 2004](#); [Wasserman et al., 2003](#); [Wasserman et al., 2000a](#); [Bellinger et al., 1994a](#); [Bellinger et al., 1994b](#); [Fergusson et al., 1993](#); [Baghurst et al., 1992](#); [Needleman et al., 1979](#)). Persistence of effects also was demonstrated by findings in some studies of rats and monkeys that gestational and/or early postnatal Pb exposures were associated with impairments in cognitive function and behavior in animals evaluated as adults ([Kuhlmann et al., 1997](#); [Altmann et al., 1993](#); [Rice, 1992b, 1990](#)). On the other hand, a few epidemiologic studies

in children ages 5-7 years demonstrated lack of persistence with findings of higher FSIQ in children who had decreases in blood Pb over time compared with children with no change or an increase in blood Pb level ([Wasserman et al., 2000a](#); [Bellinger et al., 1990](#)).

In epidemiologic studies of adults, a range of nervous system effects (e.g., decrements in memory, attention, reaction time, visuomotor tasks and reasoning, alterations in visual or brainstem evoked potentials, postural sway) were clearly indicated in Pb-exposed workers with blood Pb levels in the range of 14 to 89 µg/dL ([Iwata et al., 2005](#); [Bleecker et al., 1997](#); [Baker et al., 1979](#)). In the smaller body of studies examining nonoccupationally-exposed adults, poorer cognitive performance was associated with bone Pb levels ([Weisskopf et al., 2004](#); [Wright et al., 2003](#)) but less so with concurrent blood Pb levels ([Krieg et al., 2005](#); [Wright et al., 2003](#); [Nordberg et al., 2000](#); [Payton et al., 1998](#); [Muldoon et al., 1996](#)). These findings suggested the influence of past or cumulative Pb exposures on cognitive function decrements in nonoccupationally-exposed adults. With regard to neurodegenerative diseases, whereas a few studies of aged animals found Pb-induced amyloid plaques, a pathology commonly found in the brains of adults with Alzheimer's disease ([Basha et al., 2005](#); [Zawia and Basha, 2005](#)), epidemiologic studies did not indicate that Pb exposure was associated with Alzheimer's Disease in adults. Blood and bone Pb levels were inconsistently associated with amyotrophic lateral sclerosis (ALS) in adults in the general population; however, in some case-control studies, history of occupational Pb exposure was more prevalent among ALS cases than controls ([Kamel et al., 2002](#); [Chancellor et al., 1993](#)). Associations were reported for essential tremor and symptoms of anxiety and depression in adults, but each was examined in only a few studies.

As discussed throughout this section, recent epidemiologic and toxicological studies continued to demonstrate associations of Pb exposure and biomarkers of Pb exposure with nervous system effects. The strongest evidence continued to be derived from associations observed for Pb exposure in animals and blood Pb levels in children with cognitive function decrements. Several recent studies in children expanded the evidence for associations between concurrent blood Pb levels and attention decrements, impulsivity, and hyperactivity. Recent epidemiologic studies in adults focused primarily on cognitive function decrements but provided additional evidence for Pb-associated psychopathological effects, ALS, Parkinson's disease, and essential tremor. Recent toxicological evidence supported the effects of prenatal and postnatal Pb exposure on learning, memory, and impulsivity in animals and examined interactions between Pb exposure and stress. New or expanded areas of toxicological research related to Pb exposure included neurofibrillary tangle formation and neurodegenerative effects after early-life Pb exposures. Recent toxicological studies added to the large extant evidence base for Pb-induced effects on endpoints describing modes of action, including

neurotransmitters, synapses, glia, neurite outgrowth, the blood brain barrier, and oxidative stress. The evidence detailed in the subsequent sections continue to enhance the understanding of the spectrum of nervous system effects associated with Pb exposure.

4.3.2 Cognitive Function

In children, blood and tooth Pb levels have been linked with several measures of cognitive function that are inter-related, which makes it difficult to assess whether Pb exposure has an effect on a particular domain. Epidemiologic studies have assessed cognitive function extensively by FSIQ in children ages 3 to 17 years. FSIQ has strong psychometric properties (i.e., reliability, consistency, validity), is among the most rigorously standardized cognitive function measures, is relatively stable in school-age, and has been predictive of educational achievement and life success. In children ages 6 months to 3 years, mental development has been assessed with the Bayley Scales of Infant Development. A large body of evidence also comprises associations of blood and tooth Pb levels with memory and learning, executive function, language, and visuospatial processing. These domains of cognitive function are related to intelligence, and several are evaluated in the subtests of FSIQ. Further, some indices of memory, learning, and executive function are more comparable to endpoints examined in tests with animals. Fewer studies have examined academic performance and achievement; however, these outcomes may provide information on the impact of Pb exposure on life success. In the subsequent sections, the evidence for each of these categories of outcomes is reviewed separately, to the extent possible, in order of strength of evidence as assessed by the following parameters. Emphasis was placed on prospective epidemiologic studies with repeated measurements of blood Pb levels and cognitive function and on studies that examined blood Pb levels more similar to those of current U.S. children (i.e., $<5 \mu\text{g/dL}$) and children whose blood Pb levels were less influenced by higher past Pb exposures. Studies of chelation in children generally were not included because the high pre-chelation blood Pb levels may limit generalizability of results, and chelation itself has been linked to neurodevelopmental effects. For animal studies, emphasis was placed on studies examining Pb exposures considered relevant to this ISA, i.e., dietary exposures producing blood Pb levels $\leq 30 \mu\text{g/dL}$ ([Section 1.1](#)).

Many factors have been shown to influence the cognitive function of children, including SES, parental education, parental IQ, quality and stability of parental caregiving environment ([often measured as Home Observation for the Measurement of Environment \[HOME\]](#) [Totsika and Sylva, 2004](#)), nutritional status, and birth weight ([Nation and Gleaves, 2001](#); [Wasserman and Factor-Litvak, 2001](#)). These and other influences on neurodevelopment often are correlated with blood Pb levels. Thus, due to their

correlation with blood Pb level and causal association with outcome, these other risk factors potentially may bias or confound the associations observed between blood Pb level and indices of cognitive function. In the evaluation of the effects of Pb independent from the effects of the other risk factors, greater weight was given to studies that more extensively accounted for potential confounding in the study design or in statistical analyses. A detailed evaluation of control for potential confounding in associations between indicators of Pb exposure and neurodevelopmental effects is located in [Section 4.3.13](#).

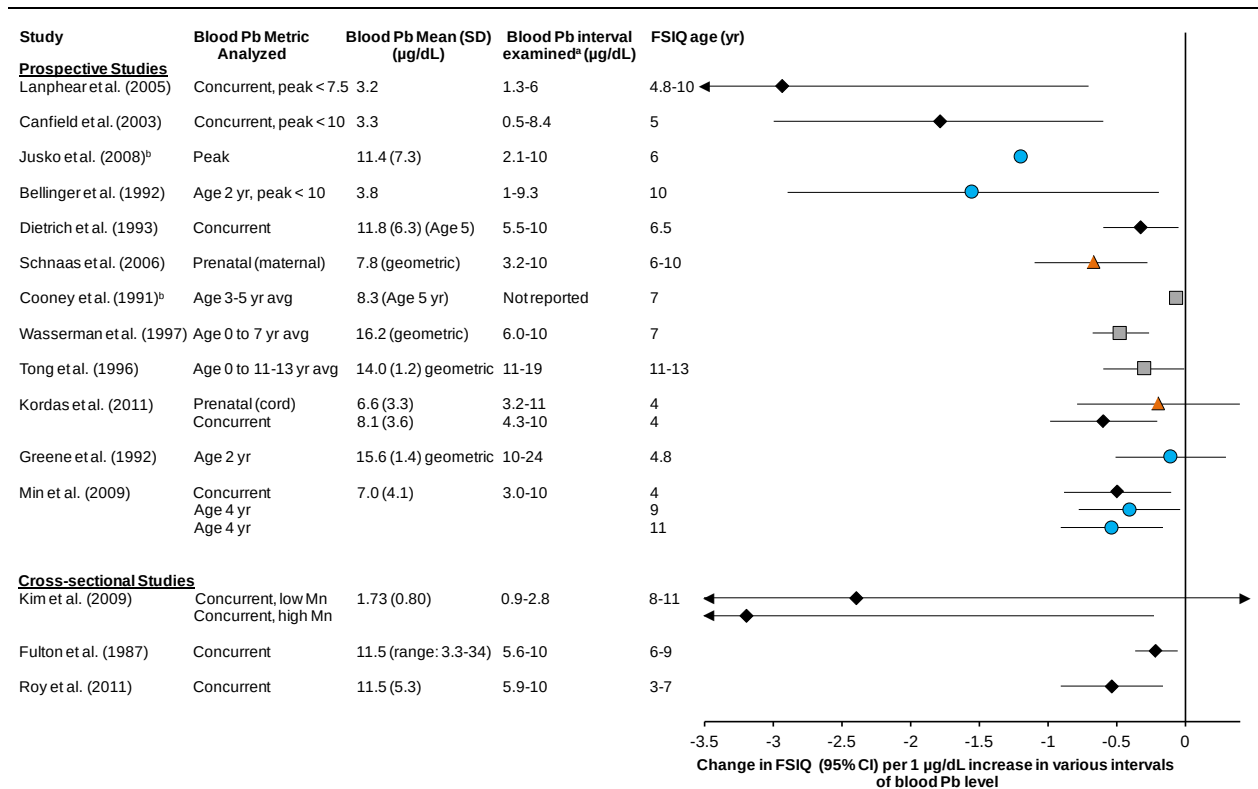
4.3.2.1 Full Scale IQ in Children

Evidence from Prospective Studies

Prospective cohort studies that were initiated in the 1980s addressed some limitations of cross-sectional studies, including better characterizing the temporal sequence between changes in blood Pb levels and cognitive function, examining the persistence of cognitive function decrements to older ages, and comparing associations among blood Pb levels measured at various lifestages or representing various time periods. Recruitment of participants before or at birth without consideration of Pb exposure or maternal IQ, high follow-up participation (>70%), and nonselective loss-to-follow-up in most studies increase confidence that the observed associations are not due to selection bias. Moreover, cooperation among investigators to adopt similar study protocols (e.g., similar tests of IQ and consideration of similar potential confounding factors) strengthened inferences regarding the consistency of associations with blood Pb level by facilitating pooled analyses and by reducing sources of heterogeneity in evaluating results across populations that varied in geographic location, proximity to Pb sources, blood Pb level range, race/ethnicity, and SES.

Individual cohort studies of varying sample sizes (n = 112-537) conducted in several different populations (e.g., Boston, MA; Cincinnati, OH; Rochester, NY; Cleveland, OH; Mexico City, Mexico; Port Pirie, Australia; and Kosovo, Yugoslavia) were consistent in demonstrating associations of higher blood Pb measured prenatally (maternal or umbilical cord) or earlier in childhood or averaged over childhood with lower FSIQ measured later in childhood, i.e., 4 to 17 years ([Schnaas et al., 2006](#); [Ris et al., 2004](#); [Canfield et al., 2003a](#); [Schnaas et al., 2000](#); [Factor-Litvak et al., 1999](#); [Tong et al., 1996](#); [Wasserman et al., 1994](#); [Dietrich et al., 1993b](#); [Baghurst et al., 1992](#); [Bellinger et al., 1992](#); [Bellinger et al., 1991](#); [McMichael et al., 1988](#)) ([Figure 4-2](#) and [Table 4-3](#)). Null or weak associations were limited to a few cohorts, namely, the Cleveland and Sydney cohorts ([Greene et al., 1992](#); [Cooney et al., 1991](#); [1989a](#); [Ernhart et al., 1988](#)). In the

prospective studies, lower FSIQ also was associated with higher concurrent blood Pb levels (Figure 4-2 and Table 4-3) and tooth Pb levels. These latter results were based on cross-sectional analyses; however, the pattern of associations consistently observed for blood Pb levels measured at various lifestages or time periods does not strongly indicate that reverse causation explains the FSIQ decrements observed in association with concurrent blood Pb or tooth Pb levels.



^aSee Table 4-3 for explanation of the blood Pb level interval examined. Effect estimates were calculated for the lowest range examined in the study or the 10th percentile of blood Pb level to a blood Pb level of 10 µg/dL.

^bSufficient data were not available to calculate 95% CI.

Note: Mn = manganese. Results are presented for most of the cohorts examined in the literature and generally are grouped according to strength of study design, representativeness of the study population characteristics and blood Pb levels examined, and extent of consideration for potential confounding. There is not necessarily a continuum of decreasing strength across studies. Results usually are presented for the oldest age examined in cohorts. Multiple results from a cohort are grouped together. To facilitate comparisons among effect estimates across studies with different distributions of blood Pb levels and model structures (e.g., linear, log-linear), effect estimates are standardized to a 1 µg/dL increase for the lowest range of blood Pb levels examined in the study or the interval from the 10th percentile of blood Pb level to 10 µg/dL. For populations with 10th percentiles near or above 10 µg/dL, the effect estimate was calculated for the 10th to 90th percentile of blood Pb level. The percentiles are estimated using various methods and are only approximate values. Effect estimates are assumed to be linear within the blood Pb level interval evaluated. The various tests used to measure FSIQ are scored on a similar scale (approximately 40-160 FSIQ points). Black diamonds, blue circles, orange triangles, and gray squares represent effect estimates for concurrent, earlier childhood, prenatal, and lifetime average blood Pb levels, respectively. The horizontal lines associated with point estimates represent 95% confidence intervals (CI).

Figure 4-2 Associations of blood Pb levels with full-scale IQ (FSIQ) in children.

Table 4-3 Additional characteristics and quantitative results for studies represented in Figure 4-2

Study	Study Population and Methodological Details ^a	Blood Pb Metric Analyzed (µg/dL)	FSIQ Testing	Effect Estimate (95% CI) ^b
Prospective Studies				
Lanphear et al. (2005) Hornung (2008)	1,333 children pooled from Boston, Cincinnati, Cleveland, Mexico City, Port Pirie, Rochester, and Yugoslavia cohorts, for each of which results are described below. Uniform analytic method applied to cohorts from diverse locations and demographic characteristics. Blood Pb levels and FSIQ measured at different ages. Several sensitivity analyses to examine heterogeneity of results by cohort, model specification, and confounding. Log linear regression model adjusted for HOME score, birth weight, maternal IQ, maternal education. Also considered potential confounding by child sex, birth order, maternal age, marital status, prenatal smoking status and alcohol use. FSIQ and covariates measured with different instruments.	Concurrent Median: 9.7 Interval analyzed: 3.3 (5 th percentile)-10	WISC-III, WISC-R, WPPSI, WISC-S Ages 4.8-10 yr	-0.82 (-1.1, -0.51)
	103 children, subset with peak blood Pb levels <7.5 µg/dL n = 13 Boston, 1 Cincinnati, 1 Cleveland, 8 Mexico City, 69 Rochester, 8 Yugoslavia, 0 Port Pirie Linear regression model adjusted for HOME score, birth weight, maternal IQ, maternal education.	Concurrent Mean: 3.2 Interval analyzed 1.3-6.0 = 5th-95th percentiles		-2.9 (-5.2, -0.71)
Canfield et al. (2003a) Canfield (2008)	101 children born 1994-1995 followed from age 6 mo to 5 yr, Rochester, NY Recruitment from study of dust control. 73% nonwhite. Moderate follow-up participation but no selective attrition. Linear regression model adjusted for child sex, Fe status, birth weight, maternal race, education, IQ, income, and prenatal smoking status, HOME score.	Concurrent, children with peak <10 Mean: 3.3 Interval analyzed: 0.5-8.4 = range Detection limit=1	Stanford-Binet Age 5 yr	-1.8 (-3.0, -0.60)
Jusko et al. (2008)	174 children born 1994-1995 followed from age 6 mo to 6 yr, Rochester, NY Same cohort as above. High follow-up participation. Higher follow-up of nonwhite, higher peak blood Pb, lower maternal IQ. Generalized additive model with LOESS adjusted for sex, birth weight, transferrin saturation, maternal race, IQ, education, and prenatal smoking status, HOME score (6 yr), family income.	Peak (6 mo-6 yr) Mean (SD): 11 (7.3) Interval analyzed: 2.1-10	WPPSI-R Age 6 yr	-1.2 ^c

Table 4-3 (Continued): Additional characteristics and quantitative results for studies represented in Figure 4-2

Study	Study Population and Methodological Details ^a	Blood Pb Metric Analyzed (µg/dL)	FSIQ Testing	Effect Estimate (95% CI) ^b
Bellinger and Needleman (2003) Bellinger (2008) U.S. EPA (2006b)	48 children followed from birth (1979-1981) to age 10 yr, Boston area, MA Recruitment at birth hospital. Participation by 59% of original cohort but 88% from age 5 yr. Participants had higher SES, HOME scores. 95% white. Linear regression model adjusted for HOME score (age 10 and 5 yr), race, maternal IQ, age, and marital status, SES, child sex, birth order, and stress events, # residence changes before age 5 yr. Also considered potential confounding by family stress, maternal age, psychiatric factors, child serum ferritin levels.	Early childhood (age 2 yr), children with peak <10 Mean: 3.8 Interval analyzed: 1-9.3 = range Detection limit not reported	WISC-R Age 10 yr	-1.6 (-2.9, -0.2)
Mazumdar et al. (2011)	43 adults followed from birth (1979-1981) to age 28-30 yr, Boston, MA area Same cohort as above. Small proportion of original cohort but no selective attrition. 93% white. Linear regression model adjusted for maternal IQ. Also found associations adjusted for maternal marital status at birth, education at birth, prenatal smoking status, or alcohol use, HOME score (mean across ages), subject sex, birth weight, birth order, gestational age, race, concussion history, or current smoking status. Also considered potential confounding by subject alcohol use.	Earlier childhood avg (age 6 mo-10 yr): NR Mean (SD), [age] 8.0 (5.3) [6 mo] 10 (6.7) [1 yr] 7.7 (4.0) [2 yr] 6.7 (3.6) [4 yr] 3.0 (2.7) [10 yr]	WASI Age 28-30 yr	-1.1 (-2.3, 0.06) ^d
Dietrich et al. (1993b)	231 children followed from birth (1979-1985) to age 6.5 yr, Cincinnati, OH Recruitment at prenatal clinic. High follow-up participation. Participants had slightly higher age 1 yr blood Pb levels. Primarily African-American. Linear regression model adjusted for HOME score, maternal IQ and prenatal smoking, child birth weight, birth length, and sex. Also considered potential confounding by perinatal complications, prenatal maternal substance abuse, and nutritional status.	Concurrent: NR Age 4-5 yr avg Mean (SD): 11.8 (6.3) Interval analyzed 5.5 (10th percentile)-10	WISC-R Age 6.5 yr	-0.33 (-0.60, -0.06)
Schnaas et al. (2006)	150 children followed from birth (1987-1992) to age 10 yr, Mexico City, Mexico. Recruitment at prenatal clinic. Low follow-up participation. Participants had higher SES, FSIQ, higher blood Pb level before age 5 yr, lower at older ages. Log linear mixed effects model adjusted for SES, maternal IQ, child sex, birth weight, postnatal blood Pb, age of 1st FSIQ test, random slope for subject. Also considered potential confounding by HOME score. Covariates assessed in pregnancy or within age 6 mo.	Prenatal (maternal 28-36 weeks) Geometric mean (5th-95th): 7.8 (2.5-25) Interval analyzed 3.2 (10th percentile)-10	WISC-R Ages 6-10 yr	-0.67 (-1.1, -0.28)

Table 4-3 (Continued): Additional characteristics and quantitative results for studies represented in Figure 4-2

Study	Study Population and Methodological Details ^a	Blood Pb Metric Analyzed (µg/dL)	FSIQ Testing	Effect Estimate (95% CI) ^b
Cooney et al. (1991)	175 children followed from birth (1983) to age 7 yr, Sydney, Australia Recruitment at birth hospital. Moderate follow-up participation but no selective attrition. 100% white. Linear regression adjusted for maternal education and IQ, paternal education and occupation, HOME score, child gestational age.	Age 3-5 yr avg: NR Age 5 yr Mean (Max): 8.3 (27)	WISC-R Age 7 yr	-0.07 (p > 0.05) ^c
Wasserman et al. (1997)	258 children followed prenatally (1984-1985) to age 7 yr, K. Mitrovica, Pristina, Yugoslavia 50% subjects live near Pb sources. Moderate follow-up participation. Participants had lower HOME score, maternal IQ, lower early childhood blood Pb levels and lived in town without Pb sources. Log linear regression adjusted for maternal age, education, and IQ, child age, sex, sibship size, and birth weight, language spoken in home, HOME score.	Lifetime avg (to age 7 yr) Geometric mean: 16 Interval analyzed 6.0 (10th percentile)-10	WISC-III Age 7 yr	-0.48 (-0.68, -0.27)
Tong et al. (1996)	375 children followed from birth (1979-1982) to age 11-13 yr, Port Pirie, Australia Residence near Pb smelter. Moderate follow-up participation. Participants had higher parental occupational prestige. Regression model adjusted for maternal IQ and age, parental occupational prestige, smoking, marital status, and education, HOME score, family functioning score, family size, child sex, age, school grade, birth weight, birth order, feeding method, breastfeeding duration, life events, prolonged absences from school. Also considered potential confounding by maternal psychopathology, child Fe status, medication use in previous 2 weeks, length of residence in area.	Lifetime avg (to age 11-13 yr) Geometric mean (GSD): 14 (1.2) Interval analyzed 11-19 = 10th-90th percentiles	WISC-R Age 11-13 yr	-0.30 (-0.60, -0.01)
Kordas et al. (2011)	186 children followed prenatally (1994-1995) to age 4 yr, Mexico City, Mexico Recruitment at prenatal clinic. Low follow-up participation but no selective attrition. Linear regression model adjusted for maternal age, education, IQ, smoking status, and marital status, crowding in home, type of floor in home, child sex, birth weight, gestational age. Did not consider potential confounding by parental caregiving quality.	Prenatal (cord) Mean (SD): 6.6 (3.3) Interval analyzed 3.2-11 = 10th-90th percentiles Concurrent Mean (SD): 8.1 (3.6) Interval analyzed 4.3 (10th percentile)-10	McCarthy GCI Age 4 yr	-0.20 (-0.79, 0.39) -0.60 (-0.99, -0.21)

Table 4-3 (Continued): Additional characteristics and quantitative results for studies represented in Figure 4-2

Study	Study Population and Methodological Details ^a	Blood Pb Metric Analyzed (µg/dL)	FSIQ Testing	Effect Estimate (95% CI) ^b
Greene et al. (1992)	270 children followed from birth (yr not reported) to age 4 yr 10 mo, Cleveland, OH Recruitment at birth hospital. High prevalence of prenatal alcohol exposure. High follow-up participation. Participants tended to be Black, prenatally exposed to marijuana. Log linear regression adjusted for maternal IQ, weight, street drug use, cigarettes/day, alcohol use, and age, parental education, authoritarian scale, race, parity, gestation duration, date of first prenatal visit, HOME score	Early childhood (age 2 yr) Geometric mean (GSD): 15.6 (1.4) Interval analyzed 10-24 = 10th-90th percentiles	WPPSI Age 4.8 yr	-0.11 (-0.51, 0.29)
Min et al. (2009)	267 children followed from birth (1994-1996) to age 11 yr, Cleveland, OH Recruitment at birth hospital. 86% African-American with high prevalence of prenatal drug and alcohol exposure. Moderate follow-up participation to age 4 yr, high retention to age 11 yr. Participants tended to be African-American and had married mothers. Linear regression model adjusted for HOME score, head circumference at birth (all ages), current caregiver vocabulary score, maternal marital status, parity, child sex (age 4 yr), maternal vocabulary score at birth (age 9 and 11 yr), race, average prenatal cocaine use (age 9 yr), prenatal 1st trimester marijuana use (age 11 yr). Also considered potential confounding by maternal education, Fe deficiency, maternal psychological distress, maternal prenatal alcohol use.	Age 4 yr Mean (SD): 7.0 (4.1) Interval analyzed 3.0 (10th percentile) -10	WPPSI, Age 4 yr concurrent	-0.50 (-0.89, -0.11)
			WISC-IV, Age 9 yr	-0.41 (-0.78, -0.04)
			WISC-IV, Age 11 yr	-0.54 (-0.91, -0.17)

Table 4-3 (Continued): Additional characteristics and quantitative results for studies represented in Figure 4-2

Study	Study Population and Methodological Details ^a	Blood Pb Metric Analyzed (µg/dL)	FSIQ Testing	Effect Estimate (95% CI) ^b
Cross-sectional Studies				
Fulton et al. (1987)	501 children, ages 6-9 yr, Edinburgh, Scotland Recruitment at schools. High participation rate, representative of area population. Log linear regression model adjusted for parental SES, education, marital status, health, mental health, cigarettes smoked, vocabulary and matrices test scores, involvement, interest, communication, and participation with child, family size and structure, child age, sex, handedness, height, gestation length, birth weight, medical history, # school absences, recent school change, grade, birth order, time of day of test, people per room in home, car/phone ownership, consumer goods ownership.	Concurrent Geometric mean (range): 12 (3.3- 34) Interval analyzed 5.6 (mean of 1st decile)-10	BASC Age 6-9 yr	-0.22 (-0.37, -0.06)
Surkan et al. (2007)	389 children, ages 6-10 years, Boston, MA, Farmington, ME Recruitment from trial of amalgam fillings. High participation rate. Higher participation of white children in Maine. Analysis of covariance adjusted for caregiver IQ, child age, SES, race, birth weight. Also considered potential confounding by site, sex, birth order, caregiver education and marital status, parenting stress, and maternal utilization of prenatal and annual health care but not parental caregiving quality.	Concurrent Group1: 1-2 Group 2: 3-4 Group 3: 5-10 Mean (SD): 2.2 (1.6)	WISC-III Age 6-10 yr	Reference -0.12 (-3.3, 3.1) ^e -6.0 (-11, -1.4) ^e
Kim et al. (2009b)	261 children (born 1996-1999) ages 8-11 yr, Seoul, Seongnam, Ulsan, and Yeoncheon, Korea Recruitment at schools. Moderate participation rate. Log linear regression model adjusted for maternal age, education and prenatal smoking status, paternal education, yearly income, smoking exposure status after birth, child age, sex, and birth weight. Did not consider potential confounding by parental caregiving quality or IQ.	Concurrent Mean (SD): 1.7 (0.80) Interval analyzed 0.9- 2.8 = 10th-90th percentiles	KEDI-WISC Ages 8-11 yr Blood Mn <1.4 µg/dL Blood Mn >1.4 µg/dL	 -2.4 (-6.0, 1.1) -3.2 (-6.1, -0.24)
Roy et al. (2011)	717 children ages 3-7 yr, Chennai, India Recruitment at schools. High participation rate. Log linear model adjusted for mid-arm circumference, age, sex, family income, parental education, family size, school, classroom. Did not consider potential confounding by parental caregiving quality.	Concurrent Mean (SD): 12 (5.3) Interval analyzed 5.9 (10th percentile)-10	Binet-Kamat Ages 3-7 yr	-0.54 (-0.91, -0.17)

Table 4-3 (Continued): Additional characteristics and quantitative results for studies represented in Figure 4-2

Study	Study Population and Methodological Details ^a	Blood Pb Metric Analyzed (µg/dL)	FSIQ Testing	Effect Estimate (95% CI) ^b
Chiodo et al. (2007)	495 children (born 1989-1991) age 7 yr, Detroit, MI area Recruitment at prenatal clinic. 100% African-American. High prevalence of prenatal drug exposure. High participation rate. Linear regression model adjusted for maternal concurrent psychopathology, IQ, prenatal cigarettes/day, and prenatal use of marijuana, SES, HOME score, caretaker education and marital status, # children in home, child sex. Also considered potential confounding by child age, maternal age, custody, cocaine use, prenatal alcohol use, and concurrent alcohol/week, cigarettes/day, and marijuana use.	Concurrent Mean (SD): 5.0 (3.0) Interval analyzed 2.1-8.7 = 10th-90th percentiles	WPPSI Age 7 yr	-0.19 (-0.30, -0.08) ^{e,f} Standardized regression coefficient
Chiodo et al. (2004)	237 children, age 7.5 yr, Detroit, MI area Recruitment at prenatal clinic. 100% African-American. High prevalence of prenatal alcohol and drug exposure. Moderate participation rate. Log linear regression model adjusted for SES, maternal education and vocabulary score, # children <18 yr, HOME score, parity, family environment scale, child sex. Also considered potential confounding by prenatal alcohol, marijuana, smoking, or cocaine use, crowding, child age and life stress, caregiver life stress, conflict tactics.	Concurrent Mean (SD): 5.4 (3.3) Interval analyzed 2.2-9.5 = 10th-90th percentiles	WISC-III Age 7.5 yr	-0.49 (-0.87, -0.12) ^{e,f} Standardized regression coefficient

WISC = Wechsler Intelligence Scale for Children, WPPSI = Wechsler Preschool and Primary Scale of Intelligence, WASI = Wechsler Abbreviated Scale of Intelligence, GCI = General Cognitive Index, BASC = British Ability Scales Combined, KEDI = Korean Educational Development Institute.

^aResults are presented for most of the cohorts examined in the literature and generally are grouped according to strength of study design, representativeness of the study population characteristics and blood Pb levels examined, and extent of consideration for potential confounding. There is not necessarily a continuum of decreasing strength across studies.

^bEffect estimates are standardized to a 1 µg/dL increase in blood Pb level within the lowest range examined in the study or 10th percentile to 10 µg/dL. For populations with 10th percentiles above 10 µg/dL, effect estimates were calculated for the 10th-90th percentile interval of blood Pb level. Effect estimates are assumed to be linear within the evaluated interval of blood Pb level. The percentiles are estimated using various methods and are only approximate values.

^cSufficient data were not available to calculate 95% CI.

^dResults not included in [Figure 4-2](#) because FSIQ assessed in adults.

^eResults not included in [Figure 4-2](#) because blood Pb level analyzed as categorical variable or because standardized regression coefficient reported.

^f95% CIs were constructed using a standard error that was estimated for the reported p-value of 0.01.

In addition to better characterizing the temporal sequence between Pb exposure and decrements in FSIQ, a common strength of most prospective studies was the adjustment for several of the potential confounding factors noted above, including maternal IQ and education, child sex and birth weight, SES, and parental caregiving quality (i.e., HOME score) ([Table 4-3](#)). Although not considered as frequently, some studies also adjusted for potential confounding by parental smoking, birth order, and nutritional factors. Multiple testing of associations between blood Pb levels and FSIQ was common in prospective studies that found and did not find associations between blood Pb level and FSIQ. However, higher probability of finding associations due to chance alone does not appear to unduly influence the evidence because in studies that found associations, there was a consistent pattern of blood Pb-associated cognitive function decrements across the various ages of blood Pb level and/or cognitive tests evaluated. Studies finding null or weak associations also tended to show a consistent pattern across the various analyses conducted.

Across cohort studies, blood Pb-associated FSIQ decrements were found in populations with mean blood Pb levels of 5 to 10 µg/dL. In analyses of the Boston cohort, Rochester cohort, and data pooled across seven cohorts restricted to children in the lower range of the blood Pb distribution (e.g., peak <10 µg/dL), associations were observed in groups of children with mean blood Pb levels of 3 to 4 µg/dL ([Bellinger, 2008](#); [Canfield, 2008](#); [Hornung, 2008](#)). Key evidence was provided by analyses of the Boston and Rochester cohorts because they had lower blood Pb levels than other cohorts examined. Further, the Rochester cohort study had extensive consideration for potential confounding by factors such as sex, family income, maternal education, race, prenatal maternal smoking, birth weight, maternal IQ, HOME score, and transferrin saturation which indicates Fe status and compared unadjusted and covariate-adjusted results ([Canfield et al., 2003a](#)). At age 5 years, higher age 6-24 month average, peak, concurrent, and lifetime average blood Pb levels (area under the curve calculated from repeat measures between age 6 months and 5 years) were associated with lower FSIQ, and while effect estimates in the covariate-adjusted model were 40-45% smaller than estimated in the unadjusted models, they remained statistically significant. While these results do not rule out potential residual confounding, they support an independent effect of Pb exposure. A larger effect was estimated for the 101 (59%) children with peak blood Pb levels < 10 µg/dL, i.e., -1.8 FSIQ points (95% CI: -3.0, -0.60) per 1 µg/dL in concurrent blood Pb level ([U.S. EPA, 2006b](#)). A larger effect also was estimated in the subset of the Boston cohort (n = 48, 32%) with peak blood Pb levels ≤10 µg/dL, i.e., -1.6 FSIQ points at age 10 years (95% CI: -2.9, -0.2) per 1 µg/dL increase in age 2-year blood Pb level. The mean blood Pb levels in these subsets of children were 3.3 (Rochester) and 3.8 µg/dL (Boston) ([Bellinger, 2008](#); [Canfield, 2008](#)) closer to that of current U.S. children compared with other prospective studies.

Analyses of the Cincinnati and Port Pirie, Australia cohorts also indicated associations between blood Pb level and FSIQ decrements with as extensive consideration for potential confounding ([Table 4-3](#)) albeit in populations with higher blood Pb levels (i.e., age 51-60 month mean: 11.8 µg/dL, lifetime [to age 11-13 years] average geometric mean: 14.0 µg/dL) ([Tong et al., 1996](#); [Dietrich et al., 1993b](#)). In contrast with other studies, in the Cleveland cohort, associations of blood Pb level (ages 2 and 3 years) and tooth Pb level with FSIQ (ages 3 and 4.8 years), were attenuated or were too imprecise to be informative with adjustment for a large number of potential confounding factors, including sociodemographic characteristics and maternal substance abuse ([Greene et al., 1992](#); [Ernhart et al., 1988](#)). In analyses of the Cleveland cohort, HOME score accounted for a large proportion of the variance in FSIQ and was the covariate that caused the greatest attenuation of the effect estimates for Pb biomarkers. The association between tooth Pb level and FSIQ at age 4.8 years was attenuated with additional adjustment for HOME score but was estimated with similar precision (-3.0 points [95% CI: -6.4, 0.32] per 1 µg/g increase in tooth Pb level) ([Greene and Ernhart, 1993](#)). The few weak or null associations do not weaken the otherwise strong evidence provided by other studies. A far larger number of other prospective studies with similar population mean blood Pb levels and important potential confounding factors considered ([Table 4-3](#)) found associations, and the Cleveland cohort had high prevalence of maternal prenatal substance abuse which may limit the external validity or representativeness of results. Further, the blood Pb-FSIQ association in children was supported by a pooled analysis of seven prospective studies ([Lanphear et al., 2005](#)), which included the Cleveland cohort, as well as multiple meta-analyses that combined results across various prospective and cross-sectional studies, including those from the Cleveland and Sydney cohorts ([Pocock et al., 1994](#); [Schwartz, 1994](#); [Needleman and Gatsonis, 1990](#)). The meta-analysis by Schwartz (1994) demonstrated the robustness of evidence to potential publication bias. The addition of eight hypothetical studies with a zero effect and with the average weight of the eight published studies resulted in a 50% lower but still negative and precise ($p < 0.001$) blood Pb-FSIQ effect estimate.

The pooled analysis of seven prospective studies (Boston, MA; Cincinnati, OH; Rochester, NY; Cleveland, OH; Mexico City, Mexico; Port Pirie, Australia; and Kosovo, Yugoslavia, individual results reported in [Figure 4-2](#) and [Table 4-3](#)) included individual-level data from 1,333 children ages 4.8-10 years with a median concurrent blood Pb level of 9.7 µg/dL (5th-95th percentile: 2.5-33.2 µg/dL) ([Lanphear et al., 2005](#)). The pooled analysis demonstrated a blood Pb-associated decrement in FSIQ in a diverse population with application of a uniform statistical model across cohorts. In multivariate models that adjusted for study site, maternal IQ, HOME score, birth weight, and maternal education, higher concurrent, peak, lifetime average, and early childhood (age 6-24 months) blood Pb levels were associated with lower FSIQ measured at age 4.8-10 years. Importantly, in

this pooled analysis and in the meta-analyses, heterogeneity among studies in the tests used to assess FSIQ and the ages of blood Pb level and FSIQ examined is a source of nondifferential measurement error that could attenuate the association to null.

Pooling data across cohorts improved characterization of the shape of the blood Pb-FSIQ concentration-response relationship at relatively low blood Pb levels. Most of the low blood Pb level data were provided by the Boston and Rochester cohorts. Consistent with the supralinear concentration-response relationship found in several individual cohort studies, a nonlinear (i.e., log-linear) model was found to fit the pooled data better than a linear model ([Lanphear et al., 2005](#); [Rothenberg and Rothenberg, 2005](#)). The nonlinear relationship was indicated further by observations of a greater decrease in FSIQ for a 1 µg/dL increase in concurrent blood Pb among the 244 (18%) children who had peak blood Pb levels <10 µg/dL (-0.80 points [95% CI: -1.7, -0.14]) and the 103 (8%) children with peak blood Pb levels <7.5 µg/dL (-2.9 points [95% CI: -5.2, -0.71]). In comparison, a log-linear analysis that included most of the study population estimated a decrease of 6.9 (95% CI: 4.2, 9.4) FSIQ points for an increase in concurrent blood Pb level from 2.4 to 30 µg/dL, which suggests a smaller FSIQ decrement per 1 µg/dL increase in blood Pb level than results from the piecewise linear models. Among children with peak blood Pb levels <10 µg/dL and <7.5 µg/dL, the mean concurrent blood Pb levels were 4.3 µg/dL and 3.2 µg/dL, respectively ([Hornung, 2008](#)).

Similar to individual cohort studies, Lanphear et al. ([2005](#)) examined several potential confounding factors related to SES and parental caregiving. HOME score, birth weight, maternal IQ, and maternal education were selected for inclusion in the final model because they were associated ($p < 0.10$) with FSIQ in a model that already contained blood Pb, or they changed the blood Pb effect estimate by more than 10%. A smaller concurrent blood Pb-associated decrement in FSIQ was estimated in the adjusted model than in the unadjusted model (-2.7 points [95% CI: -3.7, -1.7] versus -4.7 points [95% CI: -5.7, -3.6], respectively, per log increase in concurrent blood Pb level). However, the effect estimate for blood Pb level remained statistically significant. Other potential confounding factors such as child sex, maternal prenatal tobacco or alcohol use, maternal age at delivery, marital status, and birth order were not strongly associated with FSIQ ($p > 0.10$) and did not alter the effect estimate for concurrent blood Pb level adjusted for the four aforementioned covariates. Although there may be some residual confounding because there was some variation among individual studies in how they measured covariates, these results support an independent effect of Pb.

The few prospective studies published since the 2006 Pb AQCD continued to demonstrate associations between higher blood Pb level and lower FSIQ, in some cases, with additional follow-up of previous cohorts. Similar to studies reviewed in the

2006 Pb AQCD, most recent prospective studies demonstrated associations between blood Pb level and lower FSIQ in populations or smaller subgroups with mean blood Pb level 5 to 10 µg/dL. Jusko et al. (2008) examined 174 Rochester cohort subjects at age 6 years and similar to the overall results of Canfield et al. (2003a) for the cohort at age 5 years, found that the decrease in FSIQ per unit increase in peak blood Pb level was larger among children with peak blood Pb levels 2.1-10 µg/dL than among children with peak blood Pb levels 10-20 µg/dL (-1.2 points versus -0.32 points per 1 µg/dL increase in blood). The age 6 year analysis had similarly extensive consideration for potential confounding as did Canfield et al. (2003a) (Table 4-3) and also indicated associations with higher concurrent, infancy average, and lifetime average blood Pb levels (effect estimates not reported).

Additional evidence was provided for children in Mexico City, albeit in a separate cohort of children born later with lower blood Pb levels at corresponding ages. Among 150 children born 1987-1992, Schnaas et al. (2006) previously reported larger Pb-associated decrements in FSIQ for prenatal maternal (28-36 weeks) blood Pb levels than for child concurrent blood Pb levels between ages 1 and 10 years. In contrast, Kordas et al. (2011) found a larger decrement in FSIQ at age 4 years per unit increase in blood Pb level for concurrent blood Pb than cord blood Pb with adjustment for several potential confounding factors (HOME score not examined) (Table 4-3). The 186 children in the latter study were born 1994-1995 and at age 4 years had a mean blood Pb level of 8.1 µg/dL. In Schnaas et al. (2006), the geometric mean blood Pb level at age 4 years was 10.3 µg/dL. Other than mean age 4 blood Pb levels, the two Mexico City cohorts also differed in the prenatal blood Pb metric examined and age of FSIQ assessment and could have differed in the temporal patterns of Pb exposure.

Mazumdar et al. (2011) followed the Boston cohort (Bellinger et al., 1992) to age 28-30 years, and indicated that the effect of childhood Pb exposures may persist to adulthood. Only 43 of the original 249 subjects enrolled at birth were examined at age 28-30 years, but they did not differ from the original cohort in childhood blood Pb levels or demographic characteristics. Higher blood Pb levels measured at age 6 months, 4 years, 10 years, and averaged to age 10 years (means: 3 µg/dL at age 10 years to 10 µg/dL at age 1 year) were associated with lower FSIQ in adults with adjustment for one other factor: sex, birth weight, birth order, gestational age, maternal marital status, maternal education, maternal IQ, race, maternal prenatal smoking or alcohol use, childhood average HOME score, subject concussion, or subject current smoking status. The effect estimates were similar in magnitude for all childhood blood Pb metrics, except for age 6 month blood Pb level, which was associated with a smaller FSIQ decrement.

Min et al. (2009) found higher earlier childhood blood Pb levels (age 4 year) to be associated with decrements in FSIQ in another cohort of children in Cleveland, OH at ages 4, 9, and 11 years, indicating persistence of effects (Figure 4-2 and Table 4-3). But, similar to the previous Cleveland cohort (Greene et al., 1992), the recent cohort had high prevalence of prenatal alcohol and drug exposure. These exposures were weakly associated with FSIQ or did not influence the blood Pb-FSIQ association, indicating lack of strong confounding bias. However, the results may be less generalizable.

An important consideration in the evaluation of epidemiologic evidence is the precision of effect estimates, both within and among studies. There was variability in precision among studies, which did not appear to be influenced by age of subjects or the extent of adjustment for potential confounding factors. Analyses of the Port Pirie (n = 375, ages 11-13 years) (Tong et al., 1996) and Yugoslavia (n = 258, age 7 years) (Wasserman et al., 1997) cohorts and the pooled analysis of 1,333 children (Lanphear et al., 2005) estimated more precise effects compared to those of the Boston (n = 148, age 10 years) and Rochester cohorts (n = 172, age 5 years) (Canfield et al., 2003a; Bellinger et al., 1992). Analyses of the Yugoslavia and Port Pirie cohorts did not necessarily have more or less extensive adjustment for potential confounding, but the cohorts did include children living in towns with Pb smelters.

Among prospective studies, a wide range of blood Pb-associated FSIQ decrements was estimated (Figure 4-2 and Table 4-3). This wide range is not unexpected, given differences among studies in blood Pb level ranges, model specification (linear versus log linear), lifestage or time period of blood Pb examined, and distribution of potential confounding factors. The pooled analysis examined study populations of diverse SES, maternal education, and cultural backgrounds with the same model and indicated precision of effect (Lanphear et al., 2005). A series of sensitivity analyses, in which one cohort was excluded at a time, revealed that no single study was responsible for the results. Per log increase in blood Pb level, effect estimates excluding one study at a time fell within a narrow range, -2.4 to -2.9 (blood Pb level ranges for sensitivity analyses not reported). Precision of effect also was indicated by the similar effect estimates found with similar model specifications, population blood Pb levels, and sample sizes in the Boston and Rochester cohorts (Table 4-3), but very different SES and racial distributions of the cohorts, and different ages of blood Pb and FSIQ examined (Canfield et al., 2003a; Bellinger et al., 1992). These estimates were larger than those found in the Cincinnati, Port Pirie, and Yugoslavia cohorts but were based on similar extent of adjustment for potential confounding factors. Several of the smaller blood Pb-associated FSIQ decrements were based on log-linear models that estimated effects for higher blood Pb levels (e.g., estimates of 10th percentiles 5.5-12.7 versus 1.4-2.1 µg/dL). The much different null associations found in the Cleveland cohort have weaker implications

because the high prevalence of prenatal alcohol and drug exposure in the cohort may limit the representativeness of findings ([Greene et al., 1992](#); [Ernhart et al., 1988](#)).

Evidence from Cross-sectional Studies

The smaller body of cross-sectional studies reviewed in the 2006 Pb AQCD ([U.S. EPA, 2006b](#)) found associations of higher concurrent blood ([Fulton et al., 1987](#)) or tooth ([Needleman et al., 1979](#)) Pb levels with FSIQ decrements in children ages 6-9 years, and associations also were found in the few recent studies in children ages 3-11 years ([Figure 4-2](#) and [Table 4-3](#)). Several cross-sectional studies had larger sample sizes ($n = 237-717$) than the prospective studies and produced effect estimates with similar precision. Some studies had population-based recruitment, high participation rates, and did not indicate undue selection bias. Previous meta-analyses produced similar combined blood Pb-FSIQ effect estimates for prospective and cross-sectional studies ([Pocock et al., 1994](#); [Schwartz, 1994](#)). However, in this ISA, the cross-sectional findings were given less weight in conclusions regarding Pb-associated effects on cognitive function. The temporal sequence between Pb exposure and decreases in FSIQ is more difficult to establish. Among the key potential confounding factors noted at the beginning of [Section 4.3.2](#), cross-sectional studies examined potential confounding by parental education and SES, parental IQ less so, and parental caregiving quality only in a few studies ([Roy et al., 2011](#); [Kim et al., 2009b](#); [Zailina et al., 2008](#); [Surkan et al., 2007](#); [Needleman et al., 1979](#)). The meta-analysis by Pocock et al. ([1994](#)) noted the lack of adequate control for potential confounding factors in many of the cross-sectional studies evaluated.

Among the cross-sectional studies, Fulton et al. ([1987](#)) had more extensive consideration for potential confounding. Among 501 children, ages 6-9 years, in Edinburgh, Scotland, a 1 $\mu\text{g}/\text{dL}$ increase in concurrent blood Pb level in the interval between 5.6 and 10 $\mu\text{g}/\text{dL}$ was associated with a 0.22-point decrease (95% CI: -0.37, -0.06) in FSIQ, after adjustment for several factors related to SES, parental health and mental health, child health, and parental caregiving quality ([Table 4-3](#)). The effect estimate from this study was among the smallest produced by cross-sectional studies. The study population was representative of the source population but had much higher blood Pb levels (geometric mean: 11.5 $\mu\text{g}/\text{dL}$) than most current U.S. children.

Most studies that examined populations with mean concurrent blood Pb levels 1.7-5.4 $\mu\text{g}/\text{dL}$ found associations with FSIQ decrements at lower blood Pb levels. In exception, among 389 children from urban Boston, MA and rural Farmington, ME with mean concurrent blood Pb level 2.2 $\mu\text{g}/\text{dL}$, lower FSIQ was limited to children with concurrent blood Pb levels 5-10 $\mu\text{g}/\text{dL}$, with children with blood Pb levels 1-2 $\mu\text{g}/\text{dL}$ serving as the referent group ([Table 4-3](#)) ([Surkan et al., 2007](#)). There was consideration

for potential confounding by several factors, including age, race, birth weight, SES, primary caregiver IQ, SES, education and marital status, parenting stress, and maternal utilization of prenatal or annual health care. Other cross-sectional studies found associations with relatively low concurrent blood Pb levels; however, higher earlier childhood Pb exposures could have contributed to the findings. Studies in children age 7 years in Detroit, MI (mean blood Pb level ~ 5 $\mu\text{g/dL}$) found associations with adjustment for several potential confounding factors ([Chiodo et al., 2007](#); [2004](#)). Although not necessarily affecting the validity of results, the high prevalence of prenatal alcohol or drug exposure in these children may limit their generalizability. Among 261 children ages 8-11 years from four Korean cities with a mean concurrent blood Pb level 1.7 $\mu\text{g/dL}$ (detection limit=0.06 $\mu\text{g/dL}$), Kim et al. ([2009b](#)) found an association between higher concurrent blood Pb level and lower FSIQ with adjustment for parental education, yearly income, prenatal and postnatal smoking exposure, birth weight, age, sex, and blood manganese (Mn) level. The adjusted effect estimate was attenuated but similarly precise as the unadjusted estimate. The relationship of concurrent blood Pb with FSIQ and verbal IQ was modified by concurrent blood Mn levels. Blood Pb and Mn levels were not correlated ($r = 0.03$, $p = 0.64$). Higher concurrent blood Pb level was associated with lower FSIQ in both children in high Mn (above the median of 1.4 $\mu\text{g/dL}$) and low Mn group (below the median of 1.4 $\mu\text{g/dL}$) but a larger FSIQ decrement in the 130 children in the high Mn group (-3.2 points [95% CI: -6.1, -0.23] per 1 $\mu\text{g/dL}$ increase in the 10th to 90th percentile interval 0.9-2.8 $\mu\text{g/dL}$) compared with the 131 children in the low Mn group (-2.4 points [95% CI: -6.0, 1.1]). The biological plausibility for the Pb-Mn interaction is provided by observations that Mn has similar modes of action and cellular targets as does Pb, i.e., altering Ca^{2+} metabolism, inducing oxidative damage in neuronal cells, diminishing dopamine transmission. Among 169 children in Malaysia, ages 6-8 years, concurrent blood Pb (mean ~ 4 $\mu\text{g/dL}$) level was the covariate most strongly associated with FSIQ decrements ([Zailina et al., 2008](#)). Only F-statistics were reported. Family income and parental education were only weakly associated with FSIQ. The authors reported parental education to be similar between the urban and industrial study areas and at the lower secondary level. However, the variability in parental education or family income among subjects was not reported. Thus, it is not clear whether the weak association of FSIQ with parental education or family income was influenced by the lack of variability in these factors or whether they were measured inadequately. If measured inadequately, they could confound blood Pb associations.

Other recent cross-sectional studies found associations in populations of children with relatively higher concurrent blood Pb levels (means 8.1, 11.5 $\mu\text{g/dL}$). However, they did not consistently find evidence for effect modification by variants in dopamine receptor genes. Such subgroup analyses are subject to higher probability of finding an association by chance; however, the modification of Pb-associated effects on cognition by variants in

dopamine-related genes is biologically plausible. Evidence demonstrates that Pb affects dopaminergic neurons and dopamine release ([Section 4.3.10.8](#)). Further, dopaminergic activity is a key mediator of cognitive function. The larger of the studies (N = 717 children ages 3-7 years in Chennai, India) with higher concurrent blood Pb levels (mean: 11.5 µg/dL) found that a 1 µg/dL higher blood Pb level was associated with a larger decrease in FSIQ among the 73 children with the Taq A1/A1 dopamine receptor (DRD2) genotype (-1.2 points [95% CI: -2.3, -0.02] within the blood Pb level interval 6-10 µg/dL) than among the 644 children carrying the Taq A2 variant (-0.51 points [-0.92, -0.09]) ([Roy et al., 2011](#)). Kordas et al. ([2011](#)) did not find effect modification in a smaller study of 186 children age 4 years in Mexico City with a mean concurrent blood Pb level of 8.1 µg/dL. Another difference between studies that may have contributed to the difference in effect modification by the DRD2 variant was a higher mean FSIQ score observed with Taq A1/A1 genotype in the group in Mexico but no association found in the group in India.

Summary of Evidence for FSIQ

A large majority of prospective and cross-sectional studies demonstrated associations between higher blood Pb level and lower FSIQ in children ages 3-17 years (e.g., [Figure 4-2](#) and [Table 4-3](#)). While studies performed numerous tests, bias due to increased probability of finding associations by chance was unlikely because most studies found a consistent pattern of association across the ages of blood Pb level and FSIQ analyzed. Across studies, FSIQ was measured with various instruments scored on similar scales with similar measurement error. The key supporting evidence is provided by the prospective studies, which better indicated the temporal sequence between Pb exposure and FSIQ with analysis of blood Pb levels measured earlier in childhood or averaged over multiple years or tooth Pb levels and FSIQ measured later in childhood. Prospective studies also had more extensive consideration for potential confounding by maternal IQ and education, SES, birth weight, smoking exposure, parental caregiving quality, and in a few cases, other birth outcomes and nutritional factors. Further, the representativeness of findings is supported by associations found in diverse populations (e.g., Boston, MA; Cincinnati, OH; Rochester, NY; Cleveland, OH; Mexico City, Mexico; Port Pirie, Australia; and Kosovo, Yugoslavia) and studies examining populations recruited from prenatal clinics, hospital maternity departments, or schools with high follow-up participation and lack of biased follow-up participation by blood Pb level and FSIQ. A few studies found weak or null associations (i.e., Cleveland, Sydney cohorts) but did not have major strengths over other studies with respect to methodology or control for potential confounding and do not weaken the far larger body of supporting evidence.

The blood Pb-FSIQ association in children also was supported by a pooled analysis of seven prospective cohorts ([Lanphear et al., 2005](#)) as well as multiple meta-analyses that combined results across various prospective and cross-sectional studies ([Pocock et al., 1994](#); [Schwartz, 1994](#); [Needleman and Gatsonis, 1990](#)). Schwartz (1994) additionally demonstrated the robustness of evidence to potential publication bias. While supporting the robustness of the blood Pb-FSIQ association, these analyses that combine data across different populations are subject to measurement error. Nondifferential error could result from the heterogeneity in the tests used to assess FSIQ and the ages of blood Pb and FSIQ examined. There could be residual confounding due to heterogeneity among studies in the potential confounding factors examined and the method of assessment.

Across the prospective studies, blood Pb-associated FSIQ decrements at ages 4-17 years were found with concurrent, prenatal (maternal or cord), early childhood (e.g., age 2 or 4 year), multiple year average, or lifetime average blood Pb levels. Associations also were found with tooth Pb levels. There is no clear indication of an individual critical lifestage or time period of blood Pb level associated with FSIQ. Concurrent blood Pb level in children reflects recent and past Pb exposures. Thus, several observations point to an effect of cumulative Pb exposure. However, concurrent blood Pb levels tend to be correlated with those measured during other time periods.

Blood Pb-associated FSIQ decrements were found in populations with mean blood Pb levels 5-10 µg/dL. A common finding was a supralinear concentration-response relationship, i.e., a larger decrement in FSIQ per unit increase in blood Pb level in children in the lower range of the population blood Pb level distribution. In analyses restricted to children in the lower range of blood Pb levels (e.g., peak <10 µg/dL), associations were found in groups of children with mean blood Pb (concurrent or age 2 year) levels 3-4 µg/dL ([Bellinger, 2008](#); [Canfield, 2008](#); [Hornung, 2008](#)). Results from the Boston and Rochester cohorts can be considered particularly informative because within each cohort, there was relative homogeneity in the tests used to assess FSIQ, age of examination, and methods used to measure potential confounding factors. Further, in Lanphear et al. (2005), the lower portion of the blood Pb distribution largely comprised children from the Boston and Rochester cohorts.

In the pooled analysis, Lanphear et al. (2005) found a narrow range of effect estimates, -2.4 to -2.9 FSIQ points per log increase in concurrent blood Pb level, obtained by excluding one study at a time. Across individual studies, there was a wide range of effect estimates reported for blood Pb-associated FSIQ decrements. However, there was variability among studies in model specification and blood Pb level ranges examined ([Figure 4-2](#) and [Table 4-3](#)). Similarly larger effect estimates were found in the Boston and Rochester cohorts, which differed in racial and SES distributions. Although these

studies had smaller sample sizes, they had at least as extensive consideration for potential confounding as other studies ([Canfield et al., 2003a](#); [Bellinger et al., 1992](#)). Each study estimated larger effects for children whose peak blood Pb levels were less than 10 µg/dL, -1.8 FSIQ points (95% CI: -3.0, -0.60) per 1 µg/dL increase in concurrent blood Pb level in the Rochester cohort ([Canfield et al., 2003a](#)) and -1.6 points (95% CI: -2.9, -0.20) per 1 µg/dL increase in age 2 year blood Pb level in the Boston cohort ([U.S. EPA, 2006b](#); [Bellinger and Needleman, 2003](#)). These subsets of children had mean blood Pb levels of 3.3 (Rochester) ([Canfield, 2008](#)) and 3.8 µg/dL (Boston) ([Bellinger, 2008](#)), lower than those examined in other prospective studies.

4.3.2.2 Bayley Scales of Infant Development

The Mental Development Index (MDI) of the Bayley Scales of Infant Development is a widely used test of infant mental development. MDI is a reliable indicator of current development and cognitive functioning of the infant, integrating cognitive skills such as sensory/perceptual acuities, discriminations, and response; acquisition of object constancy; memory learning and problem solving; vocalization and beginning of verbal communication; and basis of abstract thinking ([McCall et al., 1972](#)). However, the MDI test is not an intelligence test, and MDI scores, particularly before ages 2-3 years, are not necessarily strongly correlated with later measurements of FSIQ in children with normal development. In the review of the MDI evidence, emphasis was placed on results from examinations at ages 2-3 years, which have test items more similar to those in school-age IQ tests. Most of the prospective studies reviewed in the 2006 Pb AQCD ([U.S. EPA, 2006b](#)) found associations of higher prenatal, earlier infancy, and concurrent blood Pb level with lower MDI score in children ages 2 to 3 years, and recent studies examined and found associations with cord blood Pb level ([Table 4-4](#)).

Table 4-4 Associations of blood Pb level with Bayley MDI in children ages 1 to 3 years.

Study	Study Population and Methodological Details Grouped by strength of study methodology, generalizability ^a	Blood Pb Metric Analyzed (µg/dL)	MDI Age and Subgroup (where analyzed)	Effect Estimate (95% CI) ^b
Bellinger et al. (1987)	182 children followed from birth (1979-1981) to age 2 yr, Boston, MA area. Prospective. Recruitment from birth hospital. High follow-up participation. Participants had higher cord blood Pb level, SES, HOME score, maternal education and IQ, lower maternal age, were white. Regression model adjusted for maternal age, race, IQ, education, years of smoking, and alcohol drinks/week in 3rd trimester, SES, HOME score, child sex, birth weight, gestational age, birth order.	Prenatal (cord) Low: <3 Medium: 6-7 High: ≥ 10	Adjusted mean at age 2 yr in High cord blood Pb group vs. Medium group vs. Low group	-3.8 (-6.3, -1.3) -4.8 (-7.3, -2.3) Concurrent reported not to be associated with MDI, quantitative data not reported
Jedrychowski et al. (2009b)	384-415 children born 2001-2004 followed prenatally to age 3 yr, Krakow, Poland Prospective cohort examining multiple exposures and outcomes. Recruitment from prenatal clinic. High follow-up participation. Log linear regression model adjusted for maternal education and prenatal smoking, child sex and birth order. Did not consider potential confounding by parenting caregiving quality.	Prenatal (cord) Median: 1.23 Detection limit not reported Interval analyzed: 0.44-5 = range < 5	Age 2 yr Age 3 yr	-1.8 (-3.4, -0.14) -1.6 (-2.9, -0.21)
Tellez-Rojo et al. (2006) Tellez-Rojo (2008)	193 children born 1994-1999 followed to age 2 yr, Mexico City, Mexico Cross-sectional. Recruitment from prenatal clinic or birth hospital. Participants had older, more educated mothers, lower cord blood Pb level, and slightly higher MDI. Linear regression model adjusted for maternal age and IQ, child sex and birth weight, cohort. Considered potential confounding by other unspecified factors.	Concurrent Median: 2.9 Detection limit not reported Interval analyzed: 0.8-4.9 = range < 5	Age 2 yr	-1.7 (-3.0, -0.42)

Table 4-4 (Continued): Associations of blood Pb level with Bayley MDI in children ages 1 to 3 years.

Study	Study Population and Methodological Details Grouped by strength of study methodology, generalizability ^a	Blood Pb Metric Analyzed (µg/dL)	MDI Age and Subgroup (where analyzed)	Effect Estimate (95% CI) ^b
Claus Henn et al. (2012)	455 children born 1997-2000 followed prenatally to age 3 yr, Mexico City, Mexico Prospective , subset of cohort above. No selective participation of subjects. Linear mixed effects regression adjusted for child sex, hemoglobin, and gestational age, maternal IQ and education, blood Pb-Mn interaction. Did not consider potential confounding by parental caregiving quality.	Age 1 yr Mean (SD): 5.1 (2.6) Interval analyzed: 2.5-8.4 =10th-90th percentiles	Ages 1 to 3 yr Blood Mn <2 µg/dL Blood Mn 2-2.8µg/dL Blood Mn >2.8 µg/dL	-2.5 -0.07 (-0.39, 0.25) -2.2 95% CIs not available for interaction model
Hu et al. (2006)	83-146 children born 1997-1999 followed prenatally to age 2 yr, Mexico City, Mexico Prospective , subset of Tellez-Rojo et al. (2006). Recruitment from prenatal clinic. Moderate follow-up participation. Eligible similar to non-eligible. Log linear regression model adjusted for maternal age and IQ, child sex, current weight, height-for-age Z score, and concurrent blood Pb (in models examining prenatal blood Pb). Considered potential confounding by other unspecified factors.	Prenatal maternal 1st trimester: Mean (SD) 7.1 (5.1) Interval analyzed 2.5 (10th percentile)-10	Age 2 yr	-0.76 (-1.5, -0.03)
		Prenatal maternal 3rd trimester: Mean (SD) 6.9 (4.2) Interval analyzed 2.8 (10th percentile)-10		-0.43 (-1.1, 0.27)
		Prenatal cord: Mean (SD) 6.2 (3.9) Interval analyzed 2.5 (10th percentile) -10		-0.06 (-0.87, 0.74)
		Concurrent: Mean (SD) 4.8 (3.7) Interval analyzed 1.6-9.1= 10th-90th percentiles		-0.23 (-0.92, 0.45)
Gomaa et al. (2002)	161-197 children followed from birth to age 2 yr, Mexico City, Mexico Prospective . Recruitment from birth hospital. Moderate participation but high retention, no selective attrition. Log linear regression model adjusted for maternal IQ, age, parents in home, breastfeeding duration, parental education, child hospitalization status and sex. Did not consider potential confounding by parental caregiving quality.	Mean (SD) Prenatal (cord): 6.7 (3.4) Interval analyzed:3.2 (10th percentile)-10	Age 2 yr	-0.82 (-1.5, -0.15)
		Concurrent: 8.4 (4.6) Interval analyzed:3.8 (10th percentile)-10		-0.01 (-0.09, 0.06)

Table 4-4 (Continued): Associations of blood Pb level with Bayley MDI in children ages 1 to 3 years.

Study	Study Population and Methodological Details Grouped by strength of study methodology, generalizability ^a	Blood Pb Metric Analyzed (µg/dL)	MDI Age and Subgroup (where analyzed)	Effect Estimate (95% CI) ^b
Pilsner et al. (2010)	221 children born 1994-1995 followed from birth to age 2 yr, Mexico City, Mexico Prospective. Recruitment from birth hospital. Low but not selective participation. Linear regression model adjusted for maternal age, IQ, marital status, and parity, gestational age, low folate intake, MTHFR genotype. Did not consider potential confounding by parental caregiving quality.	Prenatal (cord) Mean (SD): 6.7 (3.6) Interval analyzed: 3.1 (10th percentile)-10	Age 2 yr Adjust for genotype: MTHFR 1298 MTHFR 677	-0.73 (-1.2, -0.23) -0.71 (-1.2, -0.21)
Surkan et al. (2008)	309 children born 1993-2004 followed from birth to age 3 yr, Mexico City, Mexico. Cross-sectional. Recruited from prenatal or birth hospital. High participation rate. Linear mixed effects model adjusted for child sex, maternal age, IQ, education, self-esteem, parity, grams/day alcohol, smoking status, cohort. Did not consider potential confounding by parental caregiving quality.	Age 2 yr Mean (SD): 6.4 (4.3) Interval analyzed: 2.0 (10th percentile)-10	Ages 1 to 3 yr All subjects High maternal self-esteem Low maternal self-esteem	-0.18 (-0.45, 0.09) 0.36 (-0.50, 1.2) -0.31 (-0.60, -0.02)
Vimpani et al. (1985)	592 children followed prenatally to age 2 yr, Port Pirie, Australia. Prospective. Residence near Pb smelter. High baseline participation rate. Linear regression model adjusted for maternal age, education, IQ, workplace, and prenatal marital status, paternal education and workplace, parental relationship, child birth rank, mouthing activity, oxygen use at birth, apgar score, neonatal jaundice, size for gestational age. Did not consider potential confounding by parental caregiving quality.	Maternal avg prenatal: NR Cord: NR Age 6 mo: NR Age 2 yr: 20% > 30 Lifetime (to age 2 yr) avg: NR	Age 2 yr	-0.06 0.03 -0.40, p <0.05 -0.06 -0.31, p <0.05
Wasserman et al. (1992)	392 children followed prenatally to age 2 yr, K. Mitrovica, Pristina, Yugoslavia. Prospective. 53% live near Pb sources. High follow-up participation, no selective attrition. Log linear regression model adjusted for sex, birth order, birth weight, ethnic group, HOME score, maternal education, age, and IQ.	Mean (SD) Cord: 14.4 (10.4) Age 6 mo: NR Age 12 mo: NR Age 18 mo: NR Concurrent: 35 K. Mitrovica, 8.5 Pristina Geometric means	Age 2 yr	per 3-fold increase -1.7, p = 0.12 -1.1, p = 0.34 -1.7, p = 0.17 -1.8, p = 0.16 -2.5, p = 0.03

Table 4-4 (Continued): Associations of blood Pb level with Bayley MDI in children ages 1 to 3 years.

Study	Study Population and Methodological Details Grouped by strength of study methodology, generalizability ^a	Blood Pb Metric Analyzed (µg/dL)	MDI Age and Subgroup (where analyzed)	Effect Estimate (95% CI) ^b
Solon et al. (2008)	502 children born 1998-2004, Visayas, Philippines. Cross-sectional. Census based recruitment. No selective participation of subjects. Two-stage linear regression model to account for determinants of blood Pb (sex, roof material, water source, breastfed for ≥ 4 months) and cognitive function (HOME score, maternal education and smoking, born premature, region of residence, years schooling of child).	Concurrent Mean (SD): 7.1 (7.7) Interval analyzed: 1.6 (10th percentile) -10	Ages 6 mo to 3 yr	-1.07 for population mean serum folate of 225 µg/dL, 95% CI not available from model with interaction term
Ernhart et al. (1988; 1987)	146-165 children, followed prenatally to age 2 yr, Cleveland, OH Prospective. Recruitment at birth hospital. High follow-up participation. More white, higher IQ, nonalcohol using mothers not followed. 50% born to alcoholic mothers. Linear regression adjusted for age, race, sex, birth order, birth weight, parental education, maternal IQ, Authoritarian Family Ideology, HOME score.	Mean (SD) Prenatal cord: 6.0 (1.8) Age 6 mo: 10 (3.3) Concurrent: 17 (6.5)	Age 2 yr	Variance estimate 0.0003, t = -0.21 0.00, p = 0.95 0.00, p = 0.95

MDI = Mental Development Index, MTHFR = methylenetetrahydrofolate reductase

^aStudies are grouped according to strength of study design, representativeness of the population characteristics and blood Pb levels examined, and extent of consideration for potential confounding. All Mexico City studies were kept together. There is not necessarily a continuum of decreasing strength across studies.

^bExcept where noted, effect estimates were standardized to a 1 µg/dL increase in blood Pb level for the lowest blood Pb range examined in the study or for blood Pb levels up to 10 µg/dL.

The prospective studies found blood Pb-associated decrements in MDI in some large (N = 146-592) populations with mean blood Pb levels 1.3-7.1 µg/dL. Recruitment of participants before or at birth without consideration of Pb exposure or maternal IQ, high to moderate follow-up participation, and nonselective loss-to-follow-up in most studies increase confidence that the observed associations are not due to selection bias. In comparisons of blood Pb levels measured at various lifestages, some studies found a stronger association of MDI with prenatal than child postnatal blood Pb levels ([Hu et al., 2006](#); [Gomaa et al., 2002](#); [Bellinger et al., 1987](#)). Most prospective studies adjusted for birth outcomes and maternal IQ and education, and a few found associations with additional adjustment for other SES indicators, parental smoking, parental alcohol use, and parental caregiving quality. Cord blood Pb levels were associated with MDI, with additional adjustment for SES and HOME score in the Boston cohort ([Bellinger et al., 1987](#)) and HOME score in the Yugoslavia cohort ([Wasserman et al., 1992](#)). In the Cleveland cohort, associations of prenatal maternal and cord, age 6 month, and concurrent blood Pb levels with MDI at age 2 years became null after adjusting for covariates including HOME score ([Ernhart et al., 1988](#); [Ernhart et al., 1987](#)). However, about 50% of the original cohort was born to alcoholic mothers. While the high prevalence of prenatal alcohol exposure may not affect the validity of the results, it may reduce the generalizability of findings to the general U.S. population of children. Reporting only correlation coefficients, Cooney et al. ([1989b](#)) found that prenatal maternal and cord blood Pb levels (geometric means: 9.1, 8.1 µg/dL, respectively) were not associated with MDI scores in the Sydney cohort at age 2 and 3 years.

A large study of children (n = 384, 415) in Krakow, Poland, found cord blood Pb-associated decrements in MDI at ages 2 and 3 years with lower cord blood Pb levels (median 1.23 µg/dL, 5th-95th: 1.24-1.34 µg/dL) ([Jedrychowski et al., 2009b](#)) than examined in other studies ([Table 4-4](#)). However, cord blood Pb levels reflect the pregnancy blood Pb levels of mothers. Because adult blood Pb levels are influenced by past Pb exposures, there is uncertainty regarding the Pb exposure magnitude and pattern of the mothers that contributed to associations between cord blood Pb level and MDI in children. Jedrychowski et al. ([2009a](#)) estimated a larger decrease in age 3-year MDI per unit increase in cord blood Pb level among the males (51% of subjects) than among the females (49%). Other observations have indicated increased susceptibility of the developing male central nervous system (CNS) to environmental insults ([Moffitt et al., 2001](#)). Although geometric cord blood Pb levels were similar in males (1.35 µg/dL) and females (1.41 µg/dL), the mean age 3-year MDI score was slightly lower among males than among females (101 and 105, respectively).

Multiple studies in various Mexico City cohorts reported associations of prenatal (maternal or cord) or child postnatal blood Pb levels with decrements in MDI in children

between ages 1 and 3 years ([Claus Henn et al., 2012](#); [Pilsner et al., 2010](#); [Surkan et al., 2008](#); [Hu et al., 2006](#); [Téllez-Rojo et al., 2006](#)). In a subset of the cohort previously examined by Téllez-Rojo et al. (2006), Hu et al. (2006) compared associations among prenatal maternal blood Pb levels measured at different trimesters among 146 children at age 2 years. An increase in first trimester maternal blood Pb levels (whole blood or plasma) was associated with a much larger decrease in MDI score than increases in maternal third trimester, cord, or child concurrent blood Pb levels ([Table 4-4](#)). These results were adjusted for sex, age 2-year blood Pb level, height-for-age Z score, weight, maternal age, and maternal IQ. Model covariates did not include SES, maternal education, or HOME score; however, a larger unspecified list of potential confounding factors was considered in preliminary analyses.

Consistent with several findings for FSIQ, Téllez-Rojo et al. (2006) found larger effect estimates in children with lower blood Pb levels. In linear models, a 1 µg/dL increase in concurrent blood Pb level was associated with a -1.7 point (95% CI: -3.0, -0.42) change in age 2-year MDI among 193 children with concurrent blood Pb levels <5 µg/dL and a -1.0 point (95% CI: -1.8, -0.26) change among 294 children with concurrent blood Pb levels <10 µg/dL. In a follow-up of the full cohort examined in Téllez-Rojo et al. (2006) to age 3 years, Claus Henn et al. (2012) found inconsistent interactions between blood Mn and Pb levels. Investigators selected mid-range (2.0-2.8 µg/dL) blood Mn levels as the reference group based on previous observations that MDI scores were least affected by increases in blood Mn level in this group. Larger blood Pb-associated MDI decrements were found in the 91 children each with blood Mn levels <2.0 µg/dL and >2.8 µg/dL for age 1 year blood Pb level but not age 2 year blood Pb level. Kim et al. (2009b) also found effect modification by blood Mn levels for the association between blood Pb level and FSIQ, but in older children ages 8-11 years ([Section 4.3.2.1](#)).

Other recent studies in Mexico City examined effect modification by maternal self-esteem, genetic variants, and nutritional status. Surkan et al. (2008) stratified data by the level of maternal self-esteem as reported by mothers. Higher age 2-year blood Pb level was associated with lower MDI score between ages 2 and 3 years among 304 children with mothers in the lowest three quartiles of self-esteem but not among 75 children with mothers in the highest quartile of self-esteem ([Table 4-4](#)). Model covariates included cohort, sex, parity, and maternal IQ, age, education, smoking, grams/day alcohol use, and self-esteem. These findings indicated that maternal psychosocial functioning may influence the effects of Pb on the mental development of young children.

In another study in Mexico City, higher cord blood Pb level was associated with a lower MDI score in children at age 2 years (-0.73 points [95% CI: -1.2, -0.23] in MDI per 1 µg/dL increase in cord blood Pb level in a linear model) ([Pilsner et al., 2010](#)).

Investigators reported a lack of effect modification by genetic variants in the methylenetetrahydrofolate reductase (MTHFR) enzyme, which is involved in folate metabolism. The MTHFR 677 TT genotype produces an enzyme with lower metabolic activity, is associated with lower serum folate levels ([Kordas et al., 2009](#)), and in this cohort, maternal TT genotype was associated with lower mean child MDI score at age 2 years. Cord blood results from stratified analyses were not reported, thus differences in the magnitude of association between genetic variants could not be compared.

Consistent with the prospective evidence, a recent cross-sectional analysis indicated an association between higher concurrent blood Pb level and lower MDI score children in the Philippines, ages 6 months to 3 years ([Solon et al., 2008](#)). Although HOME score, premature birth, region of residence, and maternal education and prenatal smoking were examined as potential confounding factors, adjustment was made in two stages: first, adjustment for blood Pb determinants and second, adjustment for MDI determinants. This method may not adequately control for the variance shared by blood Pb level and MDI. In this cohort, children with lower folate levels had larger Pb-associated decreases in cognitive function. Among children with folate levels ≤ 230 $\mu\text{g/mL}$, 1 $\mu\text{g/dL}$ increase in blood Pb level was associated with lower MDI score in the range of -0.80 to -2.4 points. Among children with folate levels > 305 $\mu\text{g/mL}$, blood Pb level was not estimated to have a negative marginal impact. The results from this study indicated a moderating effect of folate on blood Pb level associations since folate levels were not independently associated with MDI. Higher folate level has been associated with lower blood Pb level ([Lee et al., 2005a](#)), which may be due to the role of folate in increasing Pb excretion by inhibiting the binding of Pb to blood elements ([Tandon et al., 1987](#)).

In summary, evidence consistently indicates associations of higher blood Pb levels with lower MDI scores in children ages 2-3 years ([Table 4-4](#)). Key evidence was provided by prospective studies, in particular those that adjusted for maternal IQ and education, SES, birth outcomes, and HOME score ([Wasserman et al., 1992](#); [Bellinger et al., 1987](#)). Several large studies contributed to the evidence ($N = 146\text{-}592$), and several studies had high to moderate follow-up participation and nonselective loss-to-follow-up, which reduces the likelihood of selection bias. Higher blood Pb level was associated with lower MDI scores in a few different cohorts in Mexico City and other populations, and while most adjusted for maternal education and IQ, they did not examine potential confounding by parental caregiving quality. The lack of association observed in the Cleveland cohort does not weaken the otherwise compelling evidence. The Cleveland cohort had a high prevalence of prenatal alcohol exposure, which may not affect the validity of findings but may limit the generalizability of results. Also, results from the more representative Boston and Yugoslavia studies provided supporting evidence with adjustment for several potential confounding factors. MDI at ages 2-3 years was associated with prenatal (cord

or maternal), earlier infancy, and concurrent blood Pb levels. In comparisons of blood Pb levels from different lifestages and time periods, some studies found a stronger association of MDI with prenatal than child postnatal blood Pb levels ([Hu et al., 2006](#); [Gomaa et al., 2002](#); [Bellinger et al., 1987](#)). Pb-associated decrements in MDI were found mostly in populations with mean blood Pb levels 1.3-7.1 µg/dL. A cross-sectional analysis in children in Mexico City found a larger decrement in age 2 year MDI per unit increase in concurrent blood Pb level among children with blood Pb levels <5 µg/dL versus 5-10 µg/dL or <10 µg/dL ([Téllez-Rojo et al., 2006](#)). An association was found in children in Poland with a median cord blood Pb level of 1.2 µg/dL ([Jedrychowski et al., 2009b](#)). However, since cord blood Pb levels reflect blood Pb levels of mothers, and adult blood Pb levels are influenced by past Pb exposures, the specific maternal Pb exposure magnitude and pattern contributing to the observed associations are uncertain. Overall, while evidence indicates associations of higher prenatal and postnatal child blood Pb levels with lower MDI scores in young children ages 2-3 years, the impact on later cognitive function is not certain since MDI scores do not necessarily strongly predict later IQ in children with normal development.

4.3.2.3 Learning and Memory in Children

Epidemiologic Studies of Learning and Memory in Children

The studies reviewed in the 2006 Pb AQCD did not clearly indicate associations between higher blood Pb level and poorer performance on neuropsychological tests of memory or learning (i.e., acquisition of new information) in children ages 4-17 years ([Table 4-5](#)). Studies used various tests to assess learning and memory, which may account for some of the heterogeneity observed in findings. As described in greater detail below, evidence for both memory and learning from prospective analyses in the Rochester, Boston, and Cincinnati cohorts was mixed. Previous cross-sectional studies found associations between higher concurrent blood Pb levels and poorer learning and memory, including the large (N = 4,853) study of children ages 5-16 years participating in the National Health and Nutrition Examination Survey (NHANES) III ([Lanphear et al., 2000](#)). Several recent studies, all cross-sectional, also found associations between higher concurrent blood Pb level and poorer memory in children ages 5-16 years. Some were variants of previous studies ([Krieg et al., 2010](#); [Froehlich et al., 2007](#)); others had limited implications because of little consideration for potential confounding ([Counter et al., 2008](#); [Min et al., 2007](#)).

Table 4-5 Associations between blood Pb levels and performance on tests of learning and memory in children.

Study	Study Population and Methodological Details		Blood Pb Metric Analyzed (µg/dL)	Memory/Learning Test Analyzed	Effect Estimate (95% CI) ^b
	Prospective studies presented first, then cross-sectional studies. Within each category, results are grouped by strength of study methodology, generalizability ^a				
Canfield et al. (2004)	171 children born 1994-1995 followed from age 6 mo to 5 yr, Rochester, NY Prospective. Recruitment from study of dust control. Mostly nonwhite. High follow-up participation, no selective attrition. Linear regression model adjusted for maternal education, spatial span length. Also considered potential confounding by prenatal smoking, household income, maternal IQ, ethnicity, HOME, breastfeeding duration, 1st prenatal visit, spatial working memory problem, birth weight, marital status, household crowding, neonatal intensive care unit (NICU) admission, sex, age.		Lifetime (to age 5 yr) avg Mean (SD): 7.2 (3.6) Interval analyzed: 3.5 (10th %-ile)-10	Spatial span total errors CANTAB Age 5 yr	-0.11 (-0.20, -0.02) ^c
Froehlich et al. (2007)	172-174 children born 1994-1995 followed from age 6 mo to 5 yr, Rochester, NY Cross-sectional. Same cohort as above. High follow-up participation, no selective attrition. Linear regression model adjusted for income (spatial working memory), HOME, maternal IQ, race (spatial span). Also considered potential confounding by transferrin saturation, prenatal smoking exposure, maternal education, age, NICU admission, sex.		Concurrent Mean (SD): 6.1 (4.9) Interval analyzed: 1.9 (10th %-ile)-10	Spatial working memory, total errors Spatial span length CANTAB Age 5 yr	-0.51 (-1.2, 0.16) ^c -0.02 (-0.04, 0)
Bellinger et al. (1991)	150-169 children followed from birth (1979-1981) to age 5 yr, Boston, MA area Prospective. Recruitment at birth hospital. Moderate follow-up participation. More participants were white, had higher age 2 yr HOME score, higher postnatal blood Pb levels. Log linear regression model adjusted for SES, maternal IQ and marital status, preschool attendance, HOME score, out of home care, # residence changes, medication use in previous 12 mo, # adults in home, child sex, race, birth weight, and birth order.		Mean (SD) Earlier childhood (age 2 yr): 7.0 (6.6), Interval analyzed: 1.8 (10th %-ile)-10	Memory Verbal Quantitative	-0.14 (-0.52, 0.25) -0.09 (-0.51, 0.34) -0.30 (-0.65, 0.05)
			Concurrent: 6.4 (4.1), Interval analyzed: 2.5 (10th %-ile)-10	Memory MSCA, Age 5 yr	0.09 (-0.36, 0.54)
Stiles and Bellinger (1993)	134-145 children followed from birth (1979-1981) to age 10 yr, Boston, MA area Prospective. Same cohort as above. Moderate follow-up participation, participants had higher SES and HOME score. Linear regression model		Early childhood Age 1 yr: NR Age 2 yr: NR	Perseveration score, CVLT	-0.02 (-0.04, 0) 0.03 (0.01, 0.05)

Table 4-5 (Continued): Associations between blood Pb levels and performance on tests of learning and memory in children.

Study Population and Methodological Details		Blood Pb Metric Analyzed (µg/dL)	Memory/Learning Test Analyzed	Effect Estimate (95% CI) ^b
Study	Prospective studies presented first, then cross-sectional studies. Within each category, results are grouped by strength of study methodology, generalizability ^a			
	adjusted for HOME score, maternal IQ, SES, child stress, sex and birth order, age 10 yr HOME score, maternal age, marital status, and race, # residence changes. Also considered potential confounding by family stress, birth weight, # daycare situations to age 57 mo.	Age 1 yr: NR All means reported to be <8	# trials to 1st category, WCST Age 10 yr	-0.44 (-0.93, 0.05) ^c
Ris et al. (2004)	195 children followed prenatally (1979-1985) to age 15-17 yr, Cincinnati, OH	Early childhood (age 6.5 yr)	Memory factor score of CVLT subtests	0.01 (-0.02, 0.05)
	Prospective. Recruitment at prenatal clinic. High follow-up participation, no selective attrition. Mostly African-American. Linear regression model adjusted for SES, maternal IQ, HOME, caregiver education, adolescent marijuana use, obstetrical complications, child sex and age. Also considered potential confounding by birth outcomes, maternal age, maternal prenatal smoking, alcohol, marijuana, and narcotics use, # previous abortions, stillbirths, gravidity, parity, public assistance, child health and Fe status.	Mean (SD): NR	Learning factor score of WRAT-3 and WISC-III subtests Ages 15-17 yr	-0.081 (-0.17, 0.00)
Dietrich et al. (1991)	233-236 children followed prenatally (1979-1984) to age 4 yr, Cincinnati, OH Prospective. Recruitment at prenatal clinic. High follow-up participation, no selective attrition. Mostly African-American. Linear regression adjusted for SES, birth weight, maternal IQ, prenatal marijuana use, smoking, and alcohol use, HOME, child race, preschool attendance. Also considered potential confounding by birth outcomes, maternal age and narcotics use, # previous abortions, stillbirths, gravidity, parity, caregiver education, public assistance, child age, sex, health, and Fe status.	Early childhood (Age 2 yr): NR	Achievement score KABC	0.06, p >0.05 ^d
		Concurrent: NR	Age 4 yr	0.01, p >0.05 ^d
		Lifetime avg: NR		0.07, p >0.05 ^d
Lanphear et al. (2000)	4,853 children ages 6-16 yr (born 1972-1988), U.S. NHANES III, 1988-1994 Cross-sectional. Large U.S. representative study of multiple risk factors and outcomes. Linear regression model adjusted for sex, race/ethnicity, poverty index ratio, reference adult education, serum ferritin and cotinine levels. Did not consider potential confounding by parental cognitive function or caregiving quality.	Concurrent Geometric mean (SD): 1.9 (7.0) 63.5% < 2.5 Detection limit = 0.5 Interval analyzed: 1-5	Digit Span WISC-R Ages 6-16 yr	-0.05 (-0.09, -0.01)

Table 4-5 (Continued): Associations between blood Pb levels and performance on tests of learning and memory in children.

Study Population and Methodological Details		Blood Pb Metric Analyzed (µg/dL)	Memory/Learning Test Analyzed	Effect Estimate (95% CI) ^b
Study	Prospective studies presented first, then cross-sectional studies. Within each category, results are grouped by strength of study methodology, generalizability ^a			
Krieg et al. (2010)	773 children ages 12-16 yr (born 1975-1982), U.S. NHANES III, 1991-1994 Cross-sectional. Large U.S. representative study of multiple risk factors and outcomes. Log linear regression model adjusted for sex, caregiver education, family income, race/ethnicity, test language. Did not consider potential confounding by parental cognitive function or caregiving quality.	Concurrent Mean (5th-95th): 1.95 (1.63-2.27) Detection limit = 1 Interval analyzed: 1.7-2.2 = 10th-90th percentiles	Digit span WISC-R Ages 12-16 yr	-0.34 (-0.59, -0.08)
Surkan et al. (2007)	389 children, ages 6-10 years, Boston, MA, Farmington, ME Cross-sectional. Recruitment from trial of amalgam fillings. High participation rate. Higher participation of white children in Maine. Analysis of covariance adjusted for caregiver IQ, child age, SES, race, birth weight. Also considered potential confounding by site, sex, birth order, caregiver education and marital status, parenting stress, and maternal utilization of prenatal and annual health care but not parental caregiving quality.	Concurrent Group 1: 1-2 Group 2: 3-4 Group 3: 5-10 Mean (SD): 2.2 (1.6)	General memory index, WRAML Ages 6-10 yr	Reference -0.69 (-4.4, 3.0) ^e -6.7 (-12, -1.2) ^e
Kordas et al. (2006)	293 children, ages 6-7 yr, Torreon, Mexico. Cross-sectional. Recruitment at schools. High participation rate. Residence near metal foundry. Linear regression model adjusted for child sex, age, school, birth order, hemoglobin, forgetting homework, household possessions and crowding, home ownership, maternal education, family structure, urinary arsenic, tester, school. Did not consider potential confounding by parental cognitive function or caregiving quality.	Concurrent Mean (SD): 7.1 (1.7) Interval analyzed: 2-10.0	Sternberg memory PPVT Ages 6-7 yr	-0.16 (-0.37, 0.05) -0.71 (-1.4, 0.02)

Table 4-5 (Continued): Associations between blood Pb levels and performance on tests of learning and memory in children.

Study Population and Methodological Details				
Study	Prospective studies presented first, then cross-sectional studies. Within each category, results are grouped by strength of study methodology, generalizability ^a	Blood Pb Metric Analyzed (µg/dL)	Memory/Learning Test Analyzed	Effect Estimate (95% CI) ^b
Chiodo et al. (2004)	208-236 children, age 7.5 yr, Detroit, MI area	Concurrent	Verbal learning, WRAML	-0.20, p>0.05 ^d
	Cross-sectional. Recruitment at prenatal clinic. 100% African-American. High prevalence of prenatal alcohol and drug exposure. Moderate participation rate. Log linear regression model adjusted for SES (both outcomes); caregiver vocabulary, disruption in caregiver (verbal learning); child age and sex, parity, caregiver education (spatial span). Also considered potential confounding by HOME score, maternal prenatal marijuana, smoking, alcohol, or cocaine use, crowding, child life stress, caregiver age, life stress, and psychopathology, conflict tactics, family functioning, # children <18 years.	Mean (SD): 5.4 (3.3)	Corsi Backward Spatial Span	-0.22, p>0.05 ^d
		Interval analyzed: 2.2-9.5 = 10th-90th percentiles	Age 7.5 yr	

CANTAB = Cambridge Neuropsychological Test Automated Battery, MSCA = McCarthy Scale of Children's Abilities, CVLT = California Verbal Learning Test, WCST = Wisconsin Card Sorting Test, WISC = Wechsler Intelligence Scale for Children, WRAML = Wide Range Assessment of Memory and Learning, PPVT = Peabody Picture Vocabulary Test.

^aResults are presented first for prospective studies then for cross-sectional studies. Results from the same cohort are kept together. Within each category, results are grouped by representativeness of the population characteristics and blood Pb levels examined and extent of consideration for potential confounding. There is not necessarily a continuum of decreasing strength across studies.

^bEffect estimates are standardized to a 1 µg/dL increase in blood Pb level in the lowest range of blood Pb levels examined in the study or the interval from the 10th percentile to 10 µg/dL or the 90th percentile, whichever is lower.

^cThe direction of the effect estimate was changed such that a negative estimate represents poorer performance and a positive estimate represents better performance.

^dSufficient data were not available to calculate 95% CI.

^eEffect estimate compares test performance of children in higher blood Pb groups to children in lowest blood Pb group.

The prospective studies had smaller sample sizes ($n = 134\text{--}241$) than cross-sectional studies ($n = 172\text{--}4,853$) but greater examination of potential confounding. Further, recruitment of participants before or at birth, moderate to high follow-up participation, and in most cases follow-up not biased to higher blood Pb levels and lower cognitive function reduce the likelihood of selection bias ([Table 4-5](#)). Another strength was the examination of early childhood (e.g., age 1, 2, or 6.5 year) or lifetime average (to age 5 years) blood Pb levels, which better indicated the temporal sequence between Pb exposure and decrements in learning and memory at ages 5 to 17 years.

There were contrasting associations between blood Pb levels and memory in the Rochester and Boston cohorts at age 5 years ([Canfield et al., 2004](#); [Bellinger et al., 1991](#)). The population means were similar (6.4, 7.1 $\mu\text{g/dL}$), but different blood Pb metrics were examined. In the Rochester cohort, a 1 $\mu\text{g/dL}$ increase in lifetime average blood Pb level was associated with 0.11 (95% CI: 0.02, 0.20) more total errors on the spatial span memory task (i.e., errors in replicating a sequence pattern) with adjustment for maternal age and spatial span length and consideration for several other factors ([Table 4-5](#)) ([Canfield et al., 2004](#)). Recent evidence in the Rochester cohort extended findings to associations between poorer performance on spatial working memory tasks and higher concurrent blood Pb levels ([Froehlich et al., 2007](#)), which also represent past and recent Pb exposure. In the recent analysis, associations were found with spatial span length (number of squares recalled correctly) with adjustment for HOME score, maternal IQ, and race; and with spatial working memory errors with adjustment for family income. In each analysis of the Rochester cohort, multiple associations were examined; however, there were consistent patterns of blood Pb-associated decrements in cognitive function observed across the various indices of memory, learning, and executive function examined. Coherence for associations with performance on spatial span and spatial working memory tasks was found with evidence in rodents for Pb-induced impaired performance on visual-spatial memory tasks in the Morris water maze and working memory tasks in the radial arm maze, respectively (discussed below). In the Boston cohort at age 5 years, concurrent blood Pb level was not associated with poorer memory, as assessed using the McCarthy Scale of Children's Abilities ([Bellinger et al., 1991](#)). These results were adjusted for more potential confounding factors than results from other studies, including SES, maternal IQ, and HOME score. Higher age 2 year blood Pb level was associated with poorer memory at age 5 years, but the association lacked precision ([Table 4-5](#)). Higher age 1 year blood Pb level was associated with better memory at age 10 years (i.e., fewer errors in recalling word list) as assessed with the California Verbal Learning Test ([Stiles and Bellinger, 1993](#)). In the Cincinnati cohort, higher blood Pb level was associated with improved learning at age 4 years (with age 2 year or lifetime average blood Pb) ([Dietrich et al., 1991](#)) and not associated with memory

at ages 15-17 years (with age 6.5 year blood Pb) ([Ris et al., 2004](#)) with adjustment for similar potential confounding factors plus adolescent marijuana use at ages 15-17 years.

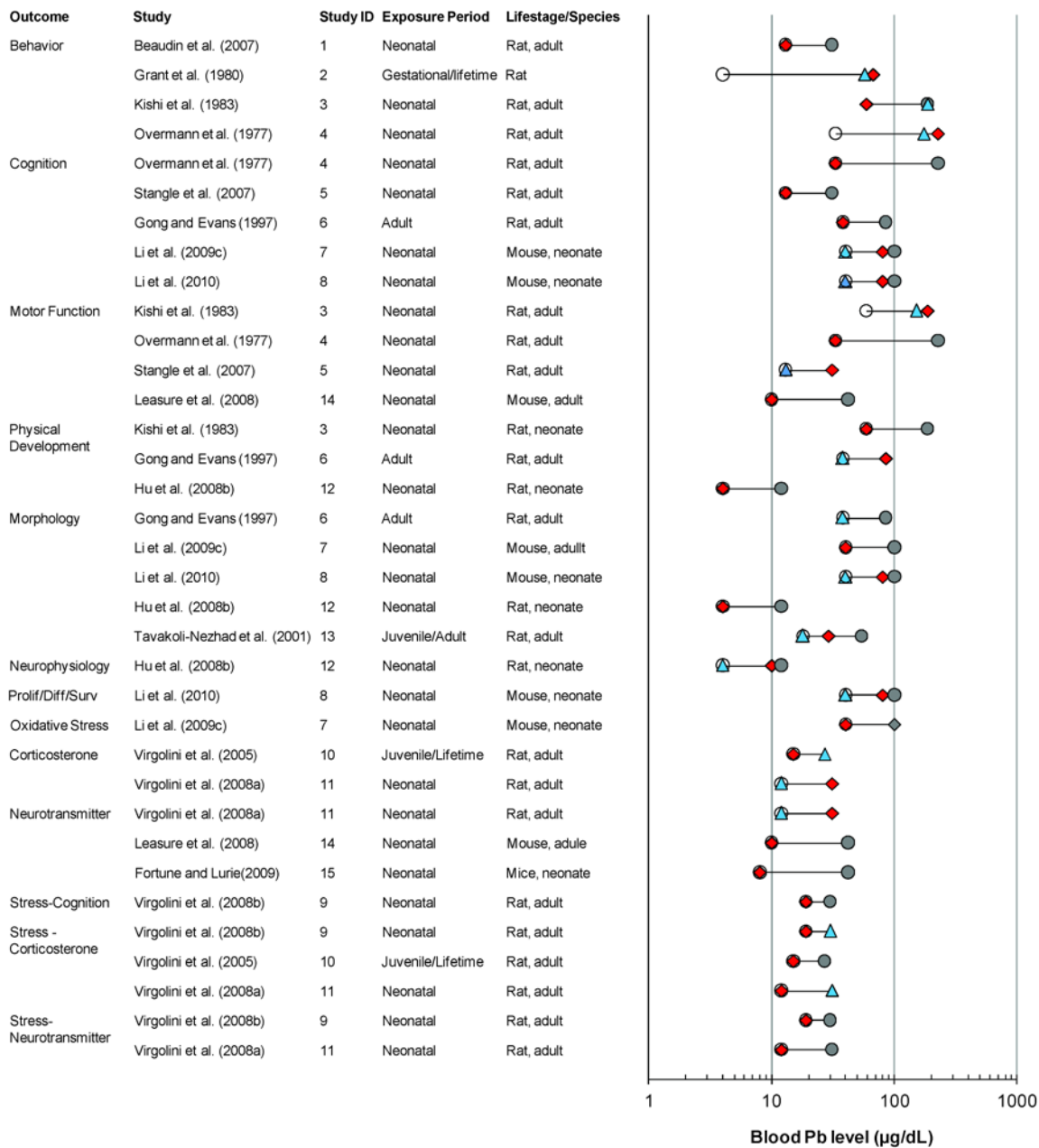
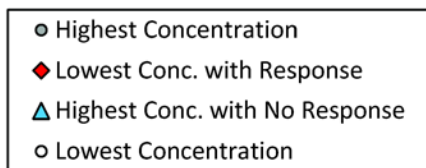
With respect to learning, associations in the Boston cohort were inconsistent across the various tests conducted, ages at which learning was assessed (ages 5, 10 years) and time periods of blood Pb levels (ages 1, 2, 5, 10 years) examined ([Stiles and Bellinger, 1993](#); [Bellinger et al., 1991](#)). In two different Boston-area cohorts examined at different ages, poorer learning (number of trials to sort cards properly or number of categories achieved) as assessed with the Wisconsin Card Sorting Test (WCST) was associated with higher age 1 year blood Pb level in children ages 10 years ([Stiles and Bellinger, 1993](#)) and with higher childhood tooth Pb levels in young adults ages 19-20 years (-0.6 categories [95% CI: -1.0, -0.21] per natural log unit increase in tooth Pb level [collected in 1st/2nd grade], with adjustment for parental IQ, maternal age and education, SES, sex, birth order, current smoking status, drug use, and alcohol use) ([Bellinger et al., 1994a](#)). In the study of 10 year-old children, poorer performance on the WCST was not consistently found in association with the various ages of blood Pb level examined ([Stiles and Bellinger, 1993](#)). Components of the WCST fall predominately in the executive function domain; however, some that measure acquisition of information also fall into the learning domain.

The cross-sectional studies examined potential confounding by parental education and SES, but a notable omission in many studies was consideration for parental caregiving quality. Chiodo et al. ([2004](#)) found concurrent blood Pb-associated decrements in spatial, verbal, and working memory with examination of HOME score. While not affecting internal validity of results, high prevalence of prenatal alcohol exposure in this cohort may limit their generalizability. HOME score was not always associated with memory. For example, in the Rochester cohort, only household income remained significantly associated with total errors in the spatial working memory task and was included in the final model ([Froehlich et al., 2007](#)). Therefore, the confounding factors may vary among endpoints. Studies found Pb-associated decrements in learning and memory in populations with mean lifetime or concurrent blood Pb levels 5.4 to 8 µg/dL. Despite the lack of information on HOME score, the cross-sectional analyses of children participating in NHANES III had several strengths, including large sample sizes (n = 773-4,853), high participation rates, lower likelihood of selection bias based specifically on Pb exposure due to the examination of multiple risk factors and outcomes, nationally-representative results, and examination of the shape of the concentration-response relationship ([Krieg et al., 2010](#); [Lanphear et al., 2000](#)). Higher concurrent blood Pb level was associated with lower digit span score in adolescents ages 12-16 years ([Krieg et al., 2010](#)) and children 6-16 years ([Lanphear et al., 2000](#)). Among children ages 6-16 years, Lanphear et al. ([2000](#)) found the largest decrement in memory score per 1 µg/dL increase in blood Pb level in children with concurrent blood Pb levels <2.5 µg/dL (-0.25 [95% CI: -0.58, 0.08])

points versus -0.05 [95% CI: -0.09, -0.01] points among all subjects). A nonlinear relationship also was found among children ages 7 years living near a metal foundry in Torreon, Mexico, with a larger Pb-associated decrement in learning and memory found in children with concurrent blood Pb levels <10 µg/dL ([Kordas et al., 2006](#)). These findings of nonlinearity based only on concurrent blood Pb without regard to early or peak childhood blood Pb levels are less certain because the magnitude and timing of Pb exposures contributing to the associations are uncertain.

Toxicological Studies of Learning and Memory

As described in the preceding sections, blood Pb levels are consistently associated with decrements in FSIQ in children but show variable associations with performance on tests of learning and memory. A relationship between Pb exposure and cognitive function decrements is supported further by evidence for Pb-induced impairments in memory and learning in animals. In the 2006 Pb AQCD ([U.S. EPA, 2006b](#)), several studies reported Pb-induced impaired memory and learning in animals with Pb exposures that resulted in blood Pb levels 30-50 µg/dL; however, some studies observed impairments in rodents (gestational exposure) and monkeys (lifetime postnatal exposure) with blood Pb levels 15-25 µg/dL ([Altmann et al., 1993](#); [Rice and Karpinski, 1988](#); [Gilbert and Rice, 1987](#)). A few recent studies added to the evidence for impaired learning processes, associative ability, or memory in animals with blood Pb levels in the range relevant to this ISA, 10-25 µg/dL ([Cory-Slechta et al., 2010](#); [Niu et al., 2009](#); [Stangle et al., 2007](#)). These blood Pb levels resulted from gestational-lactational, lactational, or lifetime (beginning in gestation or at birth) Pb exposure in drinking water. Results from animal studies on learning and other nervous system endpoints that provided concentration-response information (i.e., those with multiple Pb exposure concentrations) are shown in [Figure 4-3](#) and [Table 4-6](#). These results demonstrate the coherence among inter-related CNS changes induced by Pb exposure in animals, including deficits in CNS development and plasticity and alterations in neurotransmitters, which mediate cognitive function.



Note: The figure shows nervous system effects associated with Pb exposure in animal studies that examined multiple exposure concentrations. Doses are represented as (not necessarily peak) blood Pb levels (ID corresponds to studies described in [Table 4-6](#)).

Figure 4-3 Summary of Pb exposure-nervous system concentration-response information from animal toxicological studies.

Table 4-6 Summary of findings from neurotoxicological concentration-response array presented in Figure 4-3.

Study ID in Figure 4-3	Reference	Blood Pb Level (µg/dL)	Outcome
1	Beaudin et al. (2007)	PND52: 13 & 31	Behavior: Neonatal Pb exposure (birth to 4 weeks of age, drinking water) during and after lactation, Adult offspring showed over-reactivity to reward omission and errors in olfactory discrimination task at PND62-64, females.
2	Grant et al. (1980)	PND1: 67	Behavior: Gestational, lifetime Pb exposure (drinking water) to dams and pups, Changed locomotor development at PND14, both sexes.
3	Kishi et al. (1983)	PND22: 59	Behavior: Neonatal Pb exposure (daily oral gavage of rats PND3-21) during lactation, Changed emotional behavior at PND59-60, males.
		186	Motor function: Neonatal Pb exposure (daily oral gavage of rats PND3-21) during lactation, Motor function (rotarod performance) impaired at PND53-58, males.
		59, 152, & 186	Physical development: Neonatal Pb exposure (daily oral gavage of rats PND3-21) during lactation, Delayed development of righting reflex at PND10-17, males.
4	Overmann (1977)	PND21: 226	Behavior: Neonatal Pb exposure (oral gavage of rats PND3-21) during lactation, Aversive conditioning affected at PND26-69, both sexes.
		33, 174 & 226	Cognition: Neonatal Pb exposure (oral gavage of pups) during lactation, Response inhibition impaired at PND67-89, both sexes.
		33, 174, 226	Motor function: Neonatal Pb exposure (oral gavage of pups) during lactation, Increased nocturnal motor activity, impaired motor coordination (rotarod) at PND67-89, both sexes.
5	Stangle et al. (2007)	PND52: 13 & 31	Cognition: Neonatal Pb exposure (PND1-PND30, drinking water) during lactation, Impaired learning with visual discrimination task, heightened response to errors at PND62, both sexes.
		PND52: 31	Motor function: Neonatal Pb exposure (PND1-PND30, drinking water) during lactation, Alcove latency and response latency significantly affected at PND62, both sexes.
6	Gong and Evans (1997)	Day 21: 38 & 85	Cognition: Adult male 21 day Pb exposure (drinking water), Increased hyperactivity with habituation to new cage environment at days 21-42.
		85	Morphology: Adult male 21 day Pb exposure (drinking water), Increased hippocampal glial fibrillary acidic protein (GFAP), marker of neuronal injury.
		85	Physical development: Adult male 21 day Pb exposure (drinking water), Increased brain GFAP.

Table 4-6 (Continued): Summary of findings from neurotoxicological concentration-response array presented in Figure 4-3.

Study ID in Figure 4-3	Reference	Blood Pb Level (µg/dL)	Outcome
7	Li et al. (2009c)	PND21: 80 & 100	Cognition: Gestational & lactational Pb exposure (drinking water), Morris water maze performance impaired at PND21, both sexes.
		40, 80, & 100	Morphology: Gestational & lactational Pb exposure (drinking water), Increased levels of inflammatory cytokines & exocytosis-related proteins in brains at PND21 both sexes.
		40, 80, & 100	Oxidative stress: Gestational & lactational Pb exposure (drinking water), Elevated hippocampal levels of pro-inflammatory cytokine TNF-α in brains at PND21, both sexes.
8	Li et al. (2010b)		Cognition: Gestational & lactational Pb exposure (drinking water), Morris water maze performance impaired at PND21, both sexes
		80 & 100	Morphology: Gestational & lactational Pb exposure (drinking water), Increased hippocampal levels of P-tau and amyloid beta, Alzheimer disease-associated proteins at PND21, both sexes.
			Proliferation/differentiation/survival: Gestational & lactational Pb exposure (drinking water), Increased hippocampal expression of P-tau and amyloid beta at PND21, both sexes.
9	Virgolini et al. (2008b)	PND21: 19	Stress-induced Corticosterone: Gestational & lactational Pb exposure (drinking water, dams) plus stress, Elevated corticosterone with prenatal + offspring stress was further enhanced with Pb exposure, males.
		19 & 30	Stress-induced Cognition: Gestational & lactational Pb exposure (drinking water, dams) plus stress, Pb often has opposite effect of stress on FI performance, both sexes
		19 & 30	Stress-induced Neurotransmitter: Gestational & lactational Pb exposure (drinking water, dams) plus stress, Changes in FI performance linked to changes in dopamine and serotonin, males.
10	Virgolini et al. (2005)	Age 3 months: 15	Corticosterone: Lifetime Pb exposure (drinking water) from weaning, Pb exposure alone decreased basal plasma corticosterone levels at 5 months of age, males.
		15 & 27	Stress-induced Corticosterone: Lifetime Pb exposure (drinking water) from weaning plus cold stress increased corticosterone levels, males.
11	Virgolini et al. (2008a)	Measured in dams PND21: 31	Corticosterone: Gestational & lactational Pb exposure (maternal, drinking water), Increase in basal corticosterone levels in non-behavioral tested rats but not in behaviorally-tested rats, females at age 4-5 months.
		12	Stress-induced Corticosterone: Gestational & lactational Pb exposure (maternal, drinking water) plus maternal stress, Decreases plasma corticosterone, males behaviorally tested at age 4-5 months.
		31	Neurotransmitter: Gestational & lactational Pb exposure (maternal, drinking water), Induced NE aberrations in adult rat offspring, both sexes.
		12 & 31	Stress-induced neurotransmitter; Gestational & lactational Pb exposure (maternal, drinking water)+stress: Induced HVA (monoamine neurotransmitter metabolite) and NE aberrations in adult rat offspring, both sexes.

Table 4-6 (Continued): Summary of findings from neurotoxicological concentration-response array presented in Figure 4-3.

Study ID in Figure 4-3	Reference	Blood Pb Level (µg/dL)	Outcome
12	Hu et al. (2008b)	PND1: 4, 10 & 12	Morphology: Gestational Pb exposure (drinking water), Decreased neurite outgrowth marker PSA-NCAM in rat pups at PND1, both sexes.
		10 & 12	Neurophysiology: Gestational Pb exposure (drinking water), Decreased hippocampal sialyltransferase activity in rat pups at PND21, both sexes.
		4, 10 & 12	Physical development: Gestational Pb exposure (drinking water), Decreased neurite outgrowth marker PSA-NCAM in rat pups at PND1, both sexes.
13	Tavakoli-Nezhad et al. (2001)	Timing NR: 29, & 54	Morphology: Juvenile/adult Pb exposure (3-6 weeks, starting at PND22, drinking water), Decreased number of spontaneously active midbrain dopamine neurons at end of exposure, males
14	Leasure et al. (2008)	Peak during PND0-10: 10 & 42	Motor function: Gestational & lactational Pb exposure (maternal, drinking water), Decreased rotarod performance in offspring at age 1 year, males.
			Neurotransmitter: Gestational & lactational Pb exposure (maternal, drinking water), Affected dopamine homeostasis in offspring at age 1 year, males.
15	Fortune and Lurie (2009)	Timing NR: 8 & 42,	Neurotransmitter: Gestational & lactational Pb exposure (maternal, drinking water), Affected offspring superior olivary complex (auditory) neurotransmitters at PND21, both sexes.

Learning and Memory – Morris Water Maze

In the Rochester cohort of children, higher blood Pb level was associated with poorer performance on tests of spatial memory ([Table 4-5](#)). In animals, learning and spatial memory have been tested using the Morris water maze. These functions are assessed during training by measuring the time or distance required for a rodent to swim to a submerged platform using visual cues outside the maze. After animals have learned the location of the platform, memory for its location is assessed by removing the platform and measuring the time the animal spends in that area of the maze. The 2006 Pb AQCD ([U.S. EPA, 2006b](#)) reported mixed effects of Pb on behavior in the maze; some studies found Pb-induced impaired performance in animals whereas others found improved performance ([U.S. EPA, 2006b](#)). Evidence was more consistent for Pb-induced impairments in long-term memory (relatively permanent storage and use of unchanging information) which was found in animals with gestational, gestational/lactational, or gestation/lifetime Pb exposure producing blood Pb levels 23-33 µg/dL ([Yang et al., 2003](#); [Jett et al., 1997](#); [Kuhlmann et al., 1997](#)). Using the Morris water maze, Jett et al. ([1997](#)) found an effect in female juvenile rats of gestational/lactational Pb exposure (Pb acetate in maternal feed 10 days prior to mating to PND21) on long-term memory but not on working memory, which reflects temporary storage and manipulation of information necessary for the completion of complex tasks ([Baddeley, 1992](#)). Also using the Morris water maze, Kuhlmann et al. ([1997](#)) compared various lifestages of Pb exposure and found impaired learning and long-term memory in Long-Evans adult rats exposed to Pb during gestation and lactation (via maternal diet) or over a lifetime from gestation through adulthood. Each of these exposure periods produced peak blood Pb levels of 59 µg/dL, which are higher than those relevant to this ISA. Pb exposure during only the post-weaning period, which produced more relevant blood Pb levels of 23 and 26 µg/dL, did not affect memory.

In contrast with Kuhlmann et al. ([1997](#)), recent evidence points to impairments in memory and learning as assessed with the Morris water maze with postweaning Pb exposure, albeit with higher blood Pb levels than relevant to this ISA. Impaired learning, i.e., slower decrease in time to escape from the Morris water maze across training trials, was found in weanling Sprague-Dawley male rats exposed to 400 or 800 mg/L Pb acetate for 8 weeks in drinking water ([Fan et al., 2010](#); [Fan et al., 2009a](#)). Further, various dietary supplements (by oral gavage) in these weanling males mitigated the effect of Pb on escape latency to resemble that of control pups at the end of training. In Fan et al. ([2009a](#)), Zn and methionine given before Pb exposure prevented Pb-induced impairments in spatial memory and learning, whereas glycine, taurine, vitamin C, vitamin B1, tyrosine had no effect. In this study, blood Pb levels ranged from 7 to 70 µg/dL depending on the type and timing of nutrient supplementation and recovery time. In Fan et al. ([2010](#)),

Pb-exposed rat pups (mean blood Pb level: 50 µg/dL) showed deficits in retaining information about the platform location in probe tests with the platform removed. Methionine-choline treatment given before or concomitantly with Pb exposure prevented these impairments in spatial memory. Rats exposed to both Pb and methionine (25, 31 µg/dL) had lower blood and brain Pb levels than rats exposed only to Pb, but the results do not identify whether the protective effects of methionine are attributable to its function as a chelator and/or role as a free-radical scavenger. Choline is important for cell membranes and neurotransmitter synthesis ([Zeisel and Blusztajn, 1994](#)), and unrelated to Pb exposure, choline supplementation of rats PND16-PND30 or GD17-GD12 was shown to attenuate normal age-related declines in spatial memory ([Meck et al., 2007](#)).

Consistent with Kuhlmann et al. ([1997](#)), other recent studies found impairments in learning and memory in animals as assessed using the Morris water maze with gestational/lactational Pb exposures, albeit at higher concentrations than those relevant to this ISA. In Li et al. ([2009c](#)), rodents were exposed GD1-PND21 to Pb acetate via the drinking water of dams (1,000-10,000 ppm) and had corresponding blood Pb levels of 40-100 µg/dL at PND21. Beginning at weaning, learning and spatial memory were assessed in pups with a reversal procedure in the Morris water maze. Pb-exposed pups with blood Pb levels of 80 and 100 µg/dL had statistically significant increases in escape latency and number of platform crossings ([Li et al., 2009c](#)). Pups in Li et al. ([2009c](#)) were not separated by sex. Cao et al. ([2009](#)) found that long-term postnatal Pb exposure from birth (2,000 ppm Pb acetate in drinking water) impaired spatial memory in male Wistar rats as adults (PND81-90), and these effects were exacerbated by long-term administration of melatonin (3 mg/kg by gastric gavage, 60 days from weaning). Mechanistic support for effects on learning and memory was provided in this study by observations in the hippocampal dentate gyrus that Pb exposure also impaired long-term potentiation (LTP), a major cellular mechanism underlying learning and memory.

Working Memory – Delayed Spatial Alternation

Working memory also can be measured by testing delayed spatial alternation (DSA). In DSA, an animal receives rewards by alternating responses between two locations or levers. This test requires working memory because the correct response changes between trials, and the animal must determine which response is correct based on memory of its previous response. Impaired working memory is indicated by increased response errors, decreased percent of correct responses, and increased perseverative errors (i.e., repeatedly pressing the same lever without moving to the other lever when the reward is moved). Studies detailed in earlier Pb AQCDs showed that Pb-exposed animals had deficits in working memory as assessed with DSA ([Alber and Strupp, 1996](#); [Rice and Gilbert, 1990a](#); [Rice and Karpinski, 1988](#); [Levin et al., 1987](#)). Studies in monkeys (ages 6-9

years) showed that there were multiple lifestages and durations of Pb exposure that induced poorer performance on DSA tasks, including infancy-only, lifetime from birth, or lifetime after weaning ([Rice and Gilbert, 1990a](#); [Rice and Karpinski, 1988](#); [Levin et al., 1987](#)). Some of these effects were observed in monkeys with blood Pb levels relevant to this ISA: 15, 26 µg/dL during Pb exposure. Pb-induced impairments in DSA task performance have been observed less consistently in rats with juvenile only or juvenile to adult exposure. In fact, 8.5 month-long Pb exposure beginning at day 21 or 8 months was shown to increase accuracy of performance on DSA tasks in rats ([Cory-Slechta et al., 1991](#)).

Learning and Memory - Y Maze

The three-branch radial Y-maze test evaluates learning as the number of days required to learn the maze (90% correctly). The Y-maze has a light at the end of one of the branches. The branch with the illuminated light is the safe area whereas the other two branches are electrified and deliver a mild electric shock when entered. The spatial memory test assessed by the Y-maze test is similar to the spatial working memory test used by Froehlich et al. ([2007](#)) in the Rochester cohort, which requires children to search boxes for a reward and avoid returning to boxes where the reward was previously found. A recent study using the Y-maze showed impaired learning in Wistar albino rat offspring exposed to Pb from lactation to adulthood up to 12 weeks of age (300 mg/dL Pb acetate in dam drinking water and then in offspring drinking water postweaning) ([Niu et al., 2009](#)). Pb induced statistically significant impairments in learning at 8, 10 and 12 weeks of age but not age 6 weeks. These effects on learning were found with blood Pb levels relevant to this ISA, 17 µg/dL, as measured at age 6 weeks. Mechanistic support for Pb-induced learning impairments in this study was provided by observations of concomitant Pb-induced attenuation in levels of hippocampal glutamate ([Section 4.3.10.4](#)), which mediates signaling pathways involved in LTP.

Learning - Schedule-Controlled Behavior Testing

The 2006 Pb AQCD described the effects of Pb exposure on learning in animals as measured with operant conditioning using Fixed Interval (FI) or Fixed Ratio (FR) reinforcement schedules ([U.S. EPA, 2006b](#)) and indicated differential effects by Pb exposure concentration, with lower-level (e.g., < 10 mg/kg, blood Pb level: 11 µg/dL) and higher-level Pb (e.g., > 10 mg/kg) exposures increasing and decreasing FI response rate, respectively ([Cory-Slechta, 1994](#)). This nonlinear response has been examined further in recent work by the Cory-Slechta laboratory, much of which also examined the interaction between stress and Pb exposure. Impaired performance in FI testing with Pb exposure also supports the effects of Pb on response inhibition, which are discussed in [Section 4.3.3.1](#).

Recent evidence indicated that particular learning impairments induced by certain levels of Pb exposure were modifiable. Female rats were exposed to 300 ppm Pb acetate via dam drinking water from birth through lactation PND1-PND17 and then 20 or 30 ppm Pb via their own drinking water to PND30. Rats exposed to Pb alone (blood Pb levels at PND52: 13 and 31 µg/dL) learned the visual discrimination task more slowly than controls, indicating impaired associative ability ([Stangle et al., 2007](#)). Garavan et al. ([2000](#)) previously had similar observations in rats with gestational plus lactational Pb exposure; however, mean peak blood Pb levels were >100 µg/dL. In Stangle et al. ([2007](#)), rats subsequently administered succimer PND31-PND52 (twice daily by oral gavage, resulting in blood Pb level 2.8 µg/dL) performed better on visual discrimination tasks than did Pb-exposed rats. Brain Pb levels also were lower in the Pb+succimer group (196 ng/g) than in the two Pb-only groups (1,040 and 3,690 ng/g). In this study, succimer alone in the absence of Pb exposure resulted in some impairment in associative ability, and rats given succimer after a higher Pb concentration (30 ppm, blood Pb level 8.5 µg/dL) did not have better associative ability than rats exposed to 30 ppm Pb alone (blood Pb level: 31 µg/dL). Therefore, succimer administration did not prevent the effects of all Pb exposure on impairments in associative ability.

Learning Ability with Stress

The paradigm of combined Pb and stress exposure experienced by a laboratory animal has been examined by the Cory-Slechta laboratory, and they have focused on the common pathway of altered HPA axis and brain neurotransmitter levels. Effects on learning varied, depending on the timing of stress, Pb exposure concentration, and sex of the animal. Pb-stress interactions were found with dietary Pb exposures that resulted in blood Pb levels relevant to this ISA. Evidence additionally indicates that associations of Pb exposure and stress with learning deficits (multiple schedule of repeated learning and performance in females) may be related to aberrations in corticosterone and dopamine. As indicated in [Figure 4-3](#) and [Table 4-6](#), Pb exposure with stress has been shown to alter corticosterone levels and exacerbate Pb-induced dopamine release and learning impairments. For example, learning deficits in female rat offspring at age 2 months were enhanced following lifetime Pb exposure combined with prenatal stress, i.e., maternal restraint ([Cory-Slechta et al., 2010](#)). This exposure paradigm involved exposure of dams to 50 ppm Pb acetate from 2 months prior to mating through lactation and exposure of their pups from a mixed sex litter to 50 ppm Pb in drinking water through the remainder of their lifetime (2 months). The peak blood Pb levels measured in pups (age 5-6 days) ranged from 10 to 13 µg/dL. Sequence-specific learning deficits were found, but performance measures (e.g., accuracy, response rate) were not affected. Pb/stress was found to increase the total number of responses required to learn a response sequence. Pb/stress exposure also increased dopamine from the frontal cortex and dopamine

turnover in the nucleus accumbens, which are involved in mediating cognition. Also, Pb-exposed offspring with and without maternal stress exposure had statistically significant decreases in hippocampal nerve growth factor versus controls.

Another study of lifetime Pb exposure (50 or 150 ppm Pb acetate in drinking water of dams from 2 weeks before pregnancy through lactation and of offspring thereafter) indicated a potentiation of some effects on learning with Pb and stress co-exposure, with stress given prenatally via dams or postnatally to offspring. Effects were observed primarily in female offspring. Compared with no Pb/no stress controls, lifetime 50 ppm Pb exposure plus prenatal or postnatal stress resulting in mean blood Pb levels 11-16 µg/dL (measured at different time points) decreased the post-reinforcement pause (PRP) period in female offspring when examined starting at age 2 months ([Rossi-George et al., 2011](#)) ([Table 4-7](#), rightmost column). Animals with 150 ppm lifetime Pb exposure and mean blood Pb levels 25-33 µg/dL had decreased PRP only with prenatal stress co-exposure. Within the FI schedule, the PRP represents timing capacity or proper temporal discrimination and refers to the period during which the animal waits or pauses before depressing the lever for a reward. In this case, decreased pause or PRP interval in Pb plus stress-exposed animals indicates that they started responding earlier than did controls. These results also point to an effect of Pb on increasing impulsivity ([Section 4.3.3.1](#)). Separately, the overall FI response rate, which also indicates impulsivity (i.e., rate of not withholding responses), was significantly increased with 50 ppm lifetime Pb exposure alone and with co-exposure to maternal or offspring stress. At 150 ppm, lifetime Pb increased FI response rate only with co-exposure to stress (maternal or offspring). Biochemical analysis revealed alterations in frontal cortex norepinephrine, reductions in dopamine homeostasis in the nucleus accumbens, and enhancement of the striatal monoamine system as possible mechanistic contributions to Pb-induced impairments in learning.

Table 4-7 Summary of effects of maternal and lifetime Pb exposure on FI performance observed by Cory-Slechta laboratory.

Pb (ppm)	Maternal Pb ^b		Lifetime Pb ^c	
	Overall rate ^a	PRP ^a	Overall rate ^a	PRP ^a
0 ppm				
0-PS	No Significant Effect	No Significant Effect	No Significant Effect	No Significant Effect
0-OS	No Significant Effect	*↓ -23%	No Significant Effect	No Significant Effect
50 ppm				
50-NS	No Significant Effect	No Significant Effect	*↑ 95%	No Significant Effect
50-PS	No Significant Effect	No Significant Effect	*↑ 79.2%	*↓ -42%
50-OS	*↑ 64.9%	No Significant Effect	*↑ 74.7%	*↓ -39.3%
150 ppm				
150-NS	*↑ 42.4%	*↓ -30.3%	No Significant Effect	No Significant Effect
150-PS	No Significant Effect	*↓ -25.7%	*↑ 90.7%	*↓ -44.7%
150-OS	*↑ 59.2%	No Significant Effect	*↑ 78.5%	No Significant Effect

^aBased on calculation of group mean values across session block post-stress challenge for both maternal and lifetime Pb exposure studies. All calculations represent percent of 0-NS control values; ↑, represents increase; ↓, represents decrease.

^bData from Virgolini et al. [(2005), as reported by Rossi-George et al. (2011)].

^cData from current study, Rossi-George et al. (2011)

Notes: PRP = Post-reinforcement pause; PS = Prenatal (maternal stress); OS = Offspring stress.

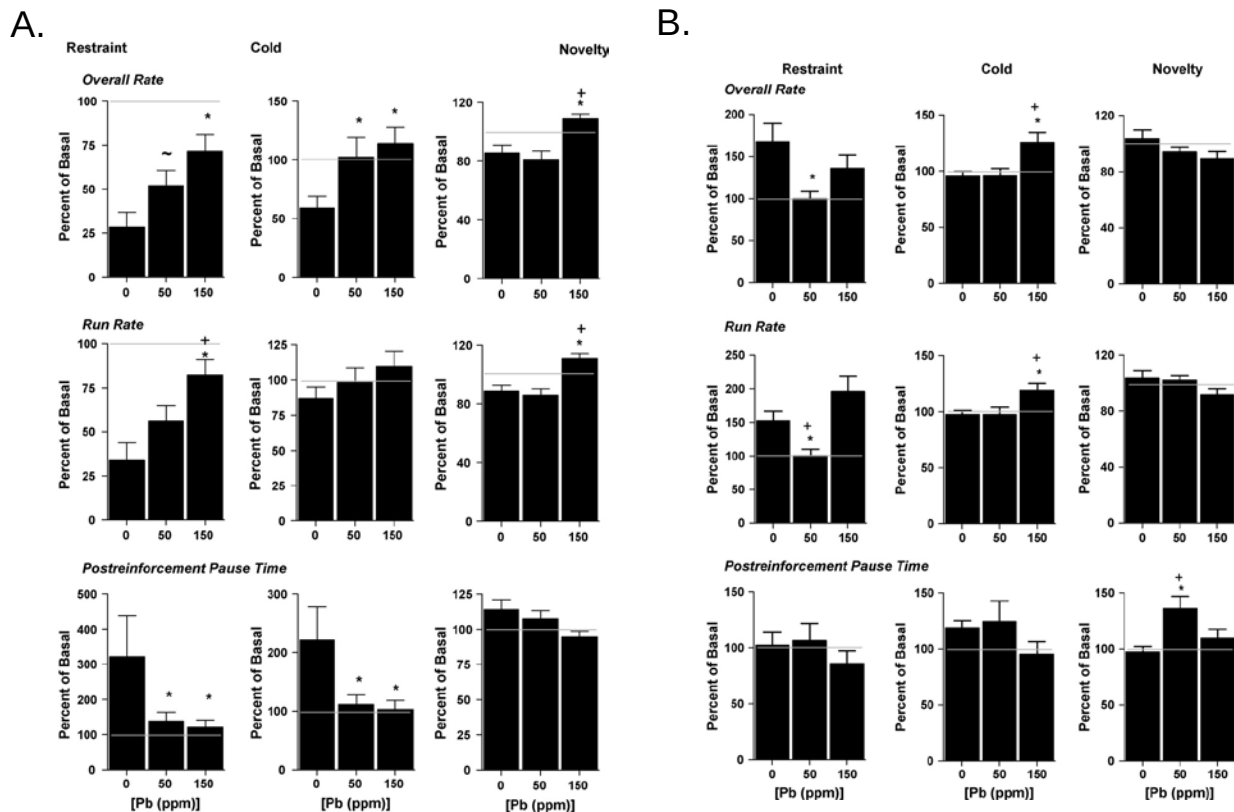
*Denotes significant effect versus 0 ppm Pb, 0 stress control (p <0.05).

Results demonstrated that lifetime 50 ppm Pb exposure (right columns) alone and with stress induced learning deficits in female rats at age 2 months as demonstrated by an increase in FI overall rate. 150 ppm Pb increased overall rate only with stress co-exposure. 50 and 150 ppm Pb decreased PRP only with stress co-exposure. Mechanistically, these authors proposed that associations of Pb and stress with learning deficits may be related to aberrations in corticosterone and dopamine. Prenatal Pb exposure alone (left columns) induced learning impairments at 150 ppm but not 50 ppm.

Source: Reprinted with permission of Elsevier Science, Table 1 of Rossi-George et al. (2011).

A separate investigation from the same laboratory similarly indicated a potentiation of effects in females with Pb and stress co-exposures but with developmental Pb exposure from two months prior to mating through lactation (50 or 150 ppm Pb acetate in drinking water) (Virgolini et al., 2008a). Dams were subject to restraint stress at GD16-GD17. Prenatal stress or Pb exposure alone did not affect FI performance in offspring. Compared with controls, marked increases in response rates on FI performance were found in female offspring at age 2-3 months with 50 ppm Pb plus prenatal and offspring stress. Using the same Pb exposure protocol, Virgolini et al. (2008b) expanded evidence for Pb-stress interactions with the examination of the effects of additional adult intermittent stress on FI performance, corticosterone, and dopamine. Adult stress was induced by placing rats in a cold 4°C room for 30 minutes or in restraints or by subjecting rats to previously unexperienced locomotor activity (novelty stress). In female offspring, adult stress induced by restraint or cold decreased overall FI response rate and increased PRP, i.e., decreased impulsivity. These effects were attenuated with 50 and 150 ppm Pb

(Figure 4-4, Panel A). In male offspring, restraint stress increased overall FI response rates and run rate. These effects were attenuated with the lower 50 ppm Pb dose (Figure 4-4, Panel B). In males, 150 ppm Pb increased overall FI response rates and run rates over those found at baseline and with stress induced by exposure to cold temperature.



Note: * denotes significantly different from 0 ppm Pb. ~denotes marginally different from 0 ppm Pb, e.g., $p = 0.07$. + denotes significantly different from the 50 ppm Pb group. Each column presents results for a particular stressor (placement in restraint, cold room, novelty) given in adulthood. In females (panel A), gestational/lactational 50 and 150 ppm Pb exposures attenuated the decreases in overall FI rate and increase in post-reinforcement-pause time induced by restraint or cold stress. In males (panel B), 50 ppm gestational/lactational Pb exposure attenuated the increase in FI overall rate and run rates induced by restraint stress.

Source: Reprinted with permission of Elsevier Science, Virgolini et al. (2008b).

Figure 4-4 Changes in Fixed Interval performance in (A) female and (B) male offspring with gestational/lactational Pb exposure plus various stressors given in adulthood.

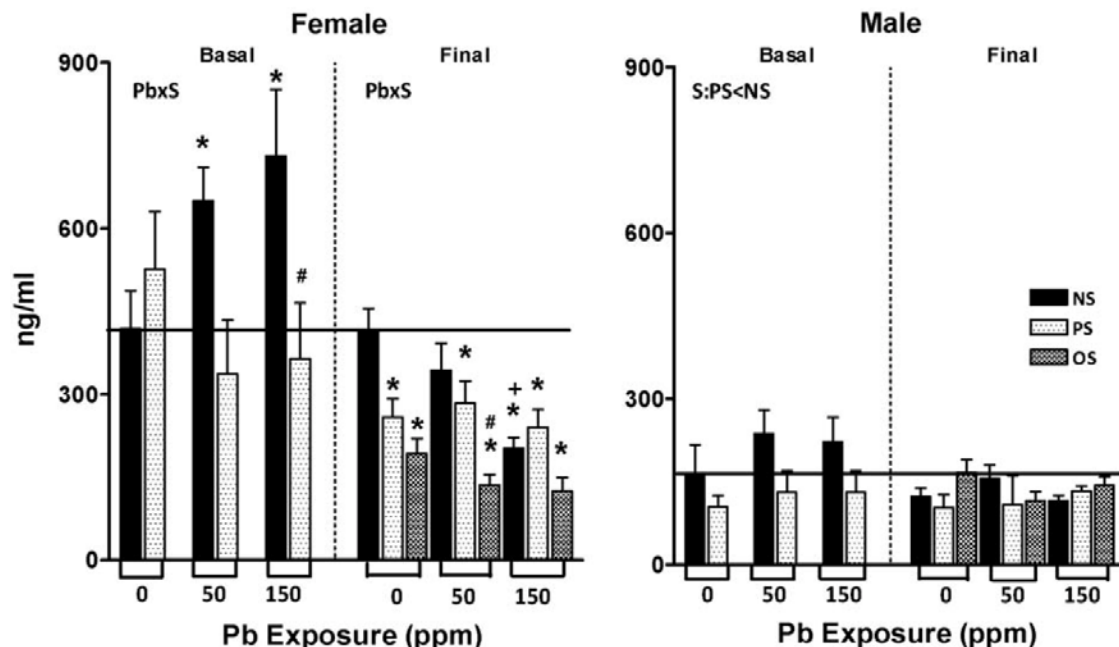
Pb exposure over various developmental windows in rodents has been shown to affect the HPA axis, as measured by the levels of corticosterone, the major glucocorticoid involved in stress responses in rodents. Thus, modulation of corticosterone may provide a

mechanistic explanation for learning deficits (FI testing in females) found with Pb and stress co-exposure in rodents. As examined by the Cory-Slechta laboratory, the effects exposure to Pb on corticosterone levels varied, depending on sex of the animal, age of the animal at assessment, and timing and duration of exposure, i.e., developmental (gestational-lactational), post-weaning, or lifetime ([Rossi-George et al., 2011](#); [Cory-Slechta et al., 2010](#); [Virgolini et al., 2008a](#)).

Because animals that are used for FI testing are regularly handled by laboratory personnel and often participate in other tests of cognition, their baseline level of stress may be skewed from that of a laboratory animal that constantly remains in a cage without daily handling. Because effects on the HPA axis are of interest to Pb researchers, the baseline corticosterone levels of animals that have participated in behavior testing (FI) and those who have not (NFI) have been compared after gestational/lactational Pb exposure. Virgolini et al. ([2008a](#)) found that baseline corticosterone levels were higher in FI than NFI animals. Also, the effect of combined gestational/lactational Pb exposure plus maternal stress on corticosterone was compared in FI and NFI animals. At the baseline age of 4-5 months, Pb exposure with or without stress did not induce differences in corticosterone levels in FI females but did in males ([Virgolini et al., 2008a](#)). In the FI males, 50 ppm Pb exposure decreased corticosterone versus control (no Pb exposure), and 150 ppm Pb exposure elevated corticosterone versus control. In male NFI animals, a U shaped concentration-response was found, with 50 ppm Pb exposure reducing corticosterone over than in the controls or with 150 ppm Pb exposure. In the NFI males, stress did not affect corticosterone levels or interact with the effect of Pb exposure. NFI females exposed to 150 ppm Pb had significantly elevated corticosterone versus control (no Pb exposure). These data demonstrate that behaviorally tested animals have altered HPA axis and altered responses to Pb exposure versus animals that are housed under conditions without daily handling by caregivers. In animals given stress as adults and not behaviorally tested, gestational-lactational Pb co-exposure prevented the stress-induced increase in corticosterone levels in females but not males, which may explain results in this study for Pb plus stress increases in FI response rates in females ([Virgolini et al., 2008b](#)).

Lifetime Pb exposure beginning in gestation (50 or 150 ppm drinking water of dams from 2 months prior to mating through lactation, then continuing in offspring water) induced increases in basal (age 2 months, before behavioral testing) corticosterone only in female offspring ([Rossi-George et al., 2011](#)) ([Figure 4-5](#)). This Pb-related increase in corticosterone at age 2 months was found in animals with blood Pb levels of 12 and 25-32 µg/dL measured at PND21. Pb-stress (either prenatal or prenatal plus offspring) interactions were observed at age 2 months but not 10 months (final, after behavioral testing). At age 2 months, Pb plus stress attenuated the Pb-induced elevations in

corticosterone to baseline levels (Figure 4-5). At 10 months of age, female offspring exposed to Pb alone or Pb plus stress had lower corticosterone concentrations than control animals. In males, corticosterone levels were not affected significantly by Pb, stress, or combined Pb/stress at 2 (basal) or 10 (final) months of age (Figure 4-5) (Rossi-George et al., 2011). In another study of lifetime Pb exposure and prenatal stress, stress alone reduced corticosterone in animals behaviorally tested at age 4 months, and Pb co-exposure blunted this decrease. In animals at age 11 months not behaviorally tested, only stress induced a decrease in corticosterone, and no interaction was found with Pb (Cory-Slechta et al., 2010). Pb-stress interactions were found in male rats exposed to Pb postweaning (PND21-age 5 months) and to stress as adults. Males exposed to 50 or 150 ppm Pb plus stress induced by placement in a cold room had higher corticosterone levels than rats exposed to Pb alone or stress alone (Virgolini et al., 2005).



Note: Corticosterone levels are noted on the y-axis. *denotes 50-NS (50 ppm Pb) or 150-NS (150 ppm Pb) is significantly different from 0-NS (no Pb, no stress) control; # denotes significantly different from corresponding Pb-NS value; + denotes significantly different from 50-NS. PS refers to prenatal dam stress, white bar with black dots. OS refers to PS followed by offspring stress, black bar with white dots. Basal measurements were taken at age 2 months, prior to the initiation of behavioral testing and final measurements were taken at 10 months, after behavioral testing. Effects of Pb and stress were observed in females not males and differed between age 2 and 10 months. At age 2 months, Pb exposure alone increased corticosterone but attenuated the prenatal stress-induced increase in corticosterone. At age 10 months, Pb exposure alone, stress exposure alone (prenatal, or prenatal- plus postnatal), and combined Pb-stress exposure decreased corticosterone.

Source: Rossi-George et al. (2011).

Figure 4-5 Mean basal and final corticosterone levels of female and male offspring exposed to lifetime Pb.

Another study examined the effects of gestational-lactational Pb acetate on the HPA axis but examined the interaction with maternal restraint stress (GD16-GD17) and offspring stress induced by injections of saline control or dexamethasone (DEX) ([Rossi-George et al., 2009](#)). Saline injection induced a spike in corticosterone followed by a time-related decline. Pb delayed and attenuated the decline in a nonlinear concentration-response in both sexes with the most profound effects found at the lower 50 ppm Pb dose. Maternal stress also prolonged the corticosterone response to saline injection and enhanced the effect of Pb in males. To test the negative feedback of the HPA axis, exogenous dexamethasone (DEX) was administered to suppress endogenous corticosterone. Pb and Pb plus maternal stress initially reduced the ability of DEX to suppress corticosterone, indicating HPA axis hypofunction. In males, with time, Pb and Pb plus stress prolonged the DEX-induced corticosterone suppression or inhibited the return to the levels in the control animals (no Pb/no stress). Rossi-George et al. ([2011](#)) additionally found that lifetime Pb significantly impacted the negative feedback by increasing nuclear glucocorticoid receptor levels. Maternal or offspring stress reduced the Pb effect in females and increased the effect of Pb in males.

To summarize the results for Pb-stress interactions in animals, Pb exposure when combined with prenatal maternal or offspring stress was found to exacerbate learning impairments compared with Pb exposure or stress alone ([Rossi-George et al., 2011](#); [Cory-Slechta et al., 2010](#); [Virgolini et al., 2008a](#)), although not uniformly across all tests or Pb doses or exposure periods. The exacerbation of learning impairments as indicated by decreases in PRP and increases in the overall response rate in performance on FI tests was found more consistently with lifetime (beginning in gestation) Pb exposure than with only gestational-lactational Pb exposure ([Table 4-7](#)). The interaction between Pb and stress may be mediated via effects on corticosterone and dopamine. Pb and stress, each given alone, have induced changes in corticosterone but not in the same direction across studies. Some but not all studies have demonstrated Pb-stress interactions on corticosterone but not always in the same direction. Gestational-lactational and lifetime Pb exposure were found to blunt the stress-induced increase in corticosterone in female rats ([Cory-Slechta et al., 2010](#); [Virgolini et al., 2008b](#)). Maternal prenatal and offspring stress were found to blunt the increase in corticosterone induced by lifetime Pb exposure ([Rossi-George et al., 2011](#)). Adult stress was found to enhance the increase in corticosterone in males induced by postweaning Pb exposure ([Virgolini et al., 2005](#)). The effects of Pb and stress on corticosterone also have differed by sex. A few studies have shown HPA axis negative feedback hypofunction with gestational-lactational and lifetime Pb exposure, with the direction of Pb-stress interactions differing between males and females ([Rossi-George et al., 2011](#); [2009](#)). The combined results show that HPA axis

alterations could provide a link for interactions found between Pb and stress in impairing learning.

4.3.2.4 Executive Function in Children

Epidemiologic evidence presented in the 2006 Pb AQCD ([U.S. EPA, 2006b](#)) indicated associations between higher childhood blood or tooth Pb levels and poorer performance on tests of executive function in children and young adults. Associations were found with indices of executive function such as strategic planning, organized search, flexibility of thought and action to a change in situation, and control of impulses (described in greater detail in [Section 4.3.3.1](#)). Prospective analyses in two Boston area and the Rochester cohorts provided key evidence with examination of blood Pb levels preceding executive function testing and adjustment for several potential confounding factors ([Canfield et al., 2004](#); [2003b](#); [Bellinger et al., 1994a](#); [Stiles and Bellinger, 1993](#)). Further, recruitment of participants before or at birth, moderate to high follow-up participation, and in most cases follow-up not biased to those with higher blood Pb levels and lower cognitive function increase confidence that the observed associations are not due to selection bias ([Table 4-8](#)). Among the few recent cross-sectional studies, most found concurrent blood Pb-associated decrements in executive function, including an analysis of the Rochester cohort ([Froehlich et al., 2007](#)). Evidence from other recent studies had weaker implications due to the limited consideration of potential confounding ([Nelson and Espy, 2009](#); [Vega-Dienstmaier et al., 2006](#)). Several of the prospective and cross-sectional studies performed multiple tests of cognitive function, including executive function. However, except for Stiles and Bellinger ([1993](#)), a consistent pattern of association was found across the various tests performed. Thus, the evidence does not appear to be biased by associations found by chance alone.

Studies in children found Pb-associated decreases in executive function using various tests including the Intra-Extra Dimensional Set Shift, WCST, and Stroop test ([Table 4-8](#)). As discussed below, studies in animals also demonstrated Pb-induced decrements in executive function, including rule learning and reversal, which also was associated with blood Pb levels in children. This coherence between findings in animals and humans for analogous outcomes further supports a relationship between Pb exposure and decrements in executive function. In both humans and animals, tests of executive function measure the ability of subjects to adjust their responses in reaction to changes in reinforcement. Poorer performance in both children and animals is indicated by more response errors, fewer correct responses, and perseverative responding (e.g., persistence in making a previously-rewarded response after a new shift in reinforcement). Biological plausibility for Pb-associated decrements in executive function also is provided by toxicological

evidence for Pb-induced changes in the availability of dopamine ([Section 4.3.10.8](#)), a neurotransmitter that affects executive functions mediated by the prefrontal cortex. Recent work unrelated to Pb shows that executive function in animals is affected by N-Methyl-D-aspartic acid or N-Methyl-D-aspartate (NMDA) receptors and dopamine-like receptors ([Herold, 2010](#)), which are two well-characterized targets of Pb.

In the Boston cohort at a relatively older age (10 years), when testing is more reliable, a 1 µg/dL increase in age 5 year blood Pb level was associated with 0.05 (95% CI: 0.01, 0.09) more perseverative errors on the WCST (errors in sorting cards according to a change in rule) ([Stiles and Bellinger, 1993](#)). In this cohort, results were inconsistent across the various cognitive tests. However, associations were more consistent for executive functions assessed by the WCST. In another cohort ages 19-20 years from towns near Boston, higher tooth Pb levels (from 1st/2nd grade) were associated with more errors on the WCST in sorting by the set rules and poorer performance on the Stroop Color and Color-word tests, which test the ability of subjects to shift focus to another dimension of stimulus that defines correct responding ([Bellinger et al., 1994a](#)).

Results from the Rochester cohort at ages 4 and 5 years indicated associations of concurrent and lifetime average blood Pb level with lower inhibition efficiency in the Shape School task (i.e., giving correct responses and withholding incorrect responses) ([Canfield et al., 2003b](#)), poorer problem solving on a spatial planning task ([Canfield et al., 2004](#)), and poorer rule learning and reversal ([Froehlich et al., 2007](#)). Associations with Shape School tasks decreased in magnitude and precision with adjustment for attention ratings, color/shape knowledge, and child IQ. These results suggest that the effect of Pb exposure on executive function may be mediated through effects on knowledge or attention. Froehlich et al. (2007) found a larger concurrent blood Pb-associated decrement in performance on a rule learning and reversal task in the Intra-Extra Dimensional Set Shift in children without the DRD4 exon III 7-repeat microsatellite (assessed using a blood Pb-DRD4-7 interaction term, $p = 0.042$). These results were unexpected, given evidence that presence of the DRD4-7 repeat is associated with reduced dopamine-induced signaling in downstream pathways (e.g., cyclic AMP), and is associated with poorer executive function in this cohort. As the results for effect modification were based on a smaller subset of subjects ($n = 116/174$), they could be due to chance. The association of concurrent blood Pb level with impaired rule learning and reversal also was greater in boys, who had lower mean scores than girls.

Table 4-8 Associations between blood or tooth Pb levels and performance on tests of executive function in children and young adults.

Study	Study Population and Methodological Details Presented first for prospective studies then for cross-sectional studies. Within each category, results are grouped by strength of study methodology, generalizability ^a	Blood Pb Metric Analyzed (µg/dL)	Executive Function Measure Analyzed	Effect Estimate (95% CI) ^b
Stiles and Bellinger (1993)	134 children followed from birth (1979-1981) to age 10 yr, Boston, MA area Prospective. Same cohort as above. Moderate follow-up participation, participants had higher SES and HOME score. Linear regression model adjusted for HOME score, maternal IQ, SES, child stress, sex and birth order, age 10 yr HOME score, family stress, marital status, race (all ages). Plus: birth weight, maternal age, # daycare situations to age 57 mo (concurrent).	Earlier childhood (age 57 mo): NR	Perseverative errors, WCST	-0.05 (-0.09, -0.01) ^c
		Concurrent: NR All means < 8	Age 10 yr	-0.05 (-0.11, 0.01) ^c
Bellinger et al. (1994a)	79 young adults followed from 1st-2nd grade (1975-1978) to ages 19-20 yr, Boston, MA area Prospective. Low follow-up participation. Participation from higher SES and initial IQ and nonwhite, but no effect on association with tooth Pb level. Log linear regression model adjusted for parental IQ, sex, SES, current drug, alcohol and illicit drug use, maternal education and age, birth order. Also considered potential confounding by other unspecified factors.	Deciduous tooth (1st/2nd grade)	Time to complete color-word test, Stroop test	-0.68 (-1.1, -0.28) ^c
		Mean (SD): 14 (11) µg/g 10th-90th: 4.3-26	Perseverative responses, WCST Ages 19-20 yr	-0.37 (-0.64, -0.10) ^c
Canfield et al. (2004)	124-151 children born 1994-1995 followed from age 6 mo to 5 yr, Rochester, NY Prospective. Recruitment from study of dust control. Mostly nonwhite. High follow-up participation, no selective attrition. Linear regression model adjusted for HOME score, maternal prenatal smoking, household income, child sex (both outcomes). Plus: NICU admission and average crowding in home (IED); maternal IQ (Stockings of Cambridge). Also considered potential confounding by breastfeeding duration, maternal ethnicity, first prenatal visit and education, spatial working memory problem, age at testing, birth weight, marital status, spatial span length.	Lifetime (to age 5 yr) avg	Stages Completed - IED Shift Task	-0.11 (-0.21, -0.01)
		Mean (SD): 7.2 (3.6) 10th-90th: 3.5-11.8	Stockings of Cambridge problems solved in minimum moves CANTAB Age 5 yr	-0.08 (-0.17, 0)

Table 4-8 (Continued): Associations between blood or tooth Pb levels and performance on tests of executive function in children and young adults.

Study Population and Methodological Details				
Study	Presented first for prospective studies then for cross-sectional studies. Within each category, results are grouped by strength of study methodology, generalizability ^a	Blood Pb Metric Analyzed (µg/dL)	Executive Function Measure Analyzed	Effect Estimate (95% CI) ^b
Froehlich et al. (2007)	174 children born 1994-1995 followed from age 6 mo to 5 yr, Rochester, NY Cross-sectional. Same cohort as above. Moderate follow-up participation, no selective attrition. Linear regression model adjusted for NICU, sex. Also considered potential confounding by income, HOME score, transferrin saturation, maternal IQ, education, prenatal smoking status, race, and age	Concurrent Mean (SD): 6.1 (4.9) 10th-90th: 1.9-11.7	Stages Completed - IED Set Shift CANTAB Age 5 yr	-0.06 (-0.12, 0)
Canfield et al. (2003b)	96-126 children born 1994-1995 followed from age 6 mo to 4-4.5 yr, Rochester, NY Cross-sectional. Same cohort as above. Moderate follow-up participation, no comparison of nonparticipants. Linear mixed effects model adjusted for child sex and age, maternal IQ, education, marital status, and prenatal smoking, household income, HOME score. Also considered potential confounding by birth order, race, gestational age, attention rating, color/shape knowledge, age 3 FSIQ.	Age 4 yr Mean: 6.5 Range: 1.7-21 10th-90th: data not available	Inhibit Efficiency (# correct-incorrect)/ phase duration) Shape School Task Repeated measures at ages 4 and 4.5 yr	-0.02 (-0.03, -0.007)
Surkan et al. (2007)	389 children, ages 6-10 years, Boston, MA, Farmington, ME Cross-sectional. Recruitment from trial of amalgam fillings. High participation rate. Higher participation of white children in Maine. Analysis of covariance adjusted for caregiver IQ, child age, SES, race, birth weight. Also considered potential confounding by site, sex, birth order, caregiver education and marital status, parenting stress, and maternal utilization of prenatal and annual health care but not parental caregiving quality.	Concurrent		
		Group 1: 1-2 Group 2: 3-4 Group 3:5-10	Perseveration errors, WCST	Reference -1.7 (-5.3, 2.0) ^{c,e} -9.2 (-15, -3.8) ^{c,e}
		Group 1: 1-2 Group 2: 3-4 Group 3:5-10	Stroop color-word interference score	Reference -0.33 (-1.9, 1.2) ^e 0.75 (-1.6, 3.1) ^e
Chiodo et al. (2004)	48 children, age 7.5 yr, Detroit, MI area Cross-sectional. Recruitment at prenatal clinic. 100% African-American High prevalence of prenatal alcohol and drug exposure. Moderate participation rate. Log linear regression model adjusted for SES, family functioning, # children <18 yr, caregiver vocabulary and education, child sex, prenatal alcohol exposure. Also considered potential confounding by HOME score, maternal prenatal marijuana, smoking, or cocaine use, crowding, child life stress, caregiver age, life stress, and psychopathology, conflict tactics, disruption in caregiver, parity, child age.	Concurrent Mean (SD): 5.4 (3.3) 10th-90th: 2.2-9.5	Perseverative errors, WCST	-0.49, p >0.05 ^{c,f}
			Percent errors Age 7.5 yr	-0.74 (-1.5, 0) ^{c,d}

Table 4-8 (Continued): Associations between blood or tooth Pb levels and performance on tests of executive function in children and young adults.

Study Population and Methodological Details				
Study	Presented first for prospective studies then for cross-sectional studies. Within each category, results are grouped by strength of study methodology, generalizability ^a	Blood Pb Metric Analyzed (µg/dL)	Executive Function Measure Analyzed	Effect Estimate (95% CI) ^b
Cho et al. (2010)	639 children ages 8-11 yr, born 1997-2000, Seoul, Seongnam, Ulsan, Incheon, Yeoncheon, Korea Cross-sectional. School-based recruitment, moderate participation rate. Log linear regression model adjusted for age, sex, paternal education, maternal IQ, child IQ, birth weight, urinary cotinine, residential area. Did not consider potential confounding by parental caregiving quality.	Concurrent Mean (SD): 1.9 (0.67) 10th-90th: 1.2-2.8 Detection limit = 0.58	Color-word score Stroop test Ages 8-11 yr	0 (-0.02, 0.02)

WCST = Wisconsin Card Sorting Test, IED = Intra-Extra Dimensional, CANTAB = Cambridge Neuropsychological Test Automated Battery.

^aResults are presented first for prospective studies then for cross-sectional studies. Within each category, results are grouped by strength of study design, representativeness of the population characteristics and blood Pb levels examined, and extent of consideration for potential confounding. There is not necessarily a continuum of decreasing strength across studies.

^bEffect estimates are standardized to a 1 µg/dL increase in blood Pb level or 1 µg/g in tooth Pb level in the 10th-90th percentile interval.

^cThe direction of the effect estimate was changed such that a negative estimate represents poorer performance.

^d95% CI was constructed using a standard error that was estimated from the reported p-value.

^eEffect estimates compare test performance of children in higher blood Pb groups to children in lowest blood Pb group.

^fSufficient data were not available to calculate 95% CI.

In addition to assessment of early childhood or cumulative Pb biomarkers, a strength of the prospective studies was the consideration for numerous potential confounding factors. The potential confounding factors varied among studies based on their association with executive function and/or influence on the Pb-executive function relationship. Some prospective studies demonstrated Pb-associated decrements in executive function with adjustment for SES, maternal IQ, and HOME score ([Canfield et al., 2004](#); [Stiles and Bellinger, 1993](#)). Others considered and excluded potential confounding by parental caregiving quality, parental smoking, maternal education, race, or birth outcomes ([Table 4-8](#)).

Blood Pb levels were reported for the Rochester cohort, and blood Pb-associated decrements in executive function were found in the cohort with mean blood Pb levels of 7.2 µg/dL for lifetime average (to age 5 years) and 6.1 and 6.5 µg/dL for concurrent (ages 4-5 years). Associations in populations with lower mean blood Pb levels (1.9-5.4 µg/dL), as assessed in cross-sectional studies with concurrent blood Pb level, were not as clearly demonstrated. While these studies adjusted for SES and parental cognitive function, most did not examine potential confounding by parental caregiving quality, i.e., HOME score. Chiodo et al. ([2004](#)) found a concurrent blood Pb-associated decrease in executive function in children ages 7 years in Detroit, MI with examination of several potential confounding factors. While not affecting internal validity, the high prevalence of prenatal alcohol exposure in the population may limit the generalizability of results. Among children in New England ages 6-10 years with mean concurrent blood Pb level 2.2 µg/dL, poorer performance on the WCST, Trail-making, and Verbal Cancellation tests was found primarily in the group with blood Pb levels 5-10 µg/dL (blood Pb group 1-2 µg/dL as reference). In this study, higher blood Pb level was not associated with poorer color-word score in the Stroop test. Cho et al. ([2010](#)) did not find a Pb-associated lower color-word score among children ages 8-11 years in five Korean cities with mean concurrent blood Pb level 1.9 µg/dL (detection limit 0.58 µg/dL). Nelson et al. ([2009](#)) found poorer executive function at age 2-6 years in children with a mean concurrent blood Pb of 6 µg/dL (compared to 2.5 µg/dL) but did not consider potential confounding and only reported F-statistics for group comparisons.

The associations observed in children between blood or tooth Pb levels and poorer executive function as assessed by the rule learning and reversal components of the Intra-Extra Dimensional Set Shift, Stroop Test, and WCST are supported by observations in animals of Pb-induced impairment in analogous measures of cognitive flexibility, tested with discrimination reversal learning and concurrent random interval (RI-RI) scheduling. The animal evidence for Pb-induced impairments in executive function was reviewed in the 2006 Pb AQCD ([U.S. EPA, 2006b](#)), and some of these observations were made with Pb exposures relevant to this ISA. Lifetime dietary Pb exposures beginning at birth or

later in infancy that produced blood Pb levels of 19-36 µg/dL were found to induce poorer performance on discrimination reversal learning tasks in monkeys ages 5-10 years ([Rice and Gilbert, 1990b](#); [Gilbert and Rice, 1987](#)). Recent work unrelated to Pb has shown that discrimination reversal learning involves NMDA receptors and dopamine-like receptors ([Herold, 2010](#)), which are two well-characterized targets of Pb. Gestational Pb exposure (blood Pb of dams >40 µg/dL) was found to impair cognitive flexibility in squirrel monkeys, ages 5-6 years, as indicated by a slower shift or lack of a shift to the lever reinforced more frequently under RI-RI scheduling ([Newland et al., 1994](#)). Rats also showed Pb-induced impairments in discrimination reversal tasks, but the authors attributed the changes to poorer associative ability instead of impaired executive function ([Garavan et al., 2000](#); [Hilson and Strupp, 1997](#)).

4.3.2.5 Academic Performance and Achievement in Children

As described in preceding sections, a large body of evidence demonstrates Pb-associated decrements in FSIQ in children, with more variable findings for performance on tests of learning and memory. Lower FSIQ and learning are linked with poorer academic performance and achievement, which may have important implications for success later in life. Further, academic performance may better assess the knowledge of an individual in the actual subject areas studied, whereas aptitude tests are used to predict future performance. In addition to FSIQ, the 2006 Pb AQCD described associations of higher blood and tooth Pb levels in children ages 5-18 years with poorer performance on tests of math, reading, and spelling skills, lower probability of high school completion and lower class rank, and lower teacher ratings of academic functioning. Associations continued to be reported in recent studies, including prospective studies examining performance on academic achievement tests and an additional analysis of adolescents participating in NHANES ([Table 4-9](#)). Multiple testing was common in studies; however, the consistent pattern of blood Pb-associated decrements in academic performance across the various tests conducted increases confidence that the evidence is not unduly biased by a higher probability of associations found by chance alone.

Table 4-9 Associations between blood or tooth Pb levels and measures of academic performance and achievement in children and young adults.

Study	Study Population and Methodological Details Organized by outcome then strength of study methodology, generalizability ^a	Blood (µg/dL) or Tooth Pb Metrics Analyzed	Measure of Academic Performance or Achievement Analyzed	Effect Estimate (95% CI) ^b
Studies of tests of academic achievement				
Chandramouli et al. (2009)	488 children followed from age 4 mo (born 1992) to age 7 yr, Avon, U.K. Prospective. All births in area eligible. Similar characteristics as U.K. census, high participation at baseline and follow-up. Participants had better educated mothers, who smoked less, better home environment. Log linear regression model adjusted for maternal education and smoking, home ownership, home facilities score, family adversity index, paternal SES, parenting attitudes at 6 mo, child sex. Also considered potential confounding by child IQ.	Earlier childhood (age 30 mo) Mean (SD): NR	Standardized Achievement Test Age 7 yr	Per doubling blood Pb -0.3 (-0.5, -0.1)
Miranda et al. (2009)	57,568 children, 4th grade, all counties, NC Prospective. Based on data from surveillance databases. 39% state test scores linked to blood Pb data. Regression model adjusted for sex, age of blood Pb measurement, race, enrollment in free/reduced lunch program, parental education, charter school. Did not consider potential confounding by parental caregiving quality or cognitive function.	Earlier childhood (9-36 mo) Median: 4.8 25th-75th: 3-6 Detection limit = 1	Reading 4th grade end-of-grade test score	Score vs. blood Pb category 1 µg/dL 2 µg/dL: -0.30 (-0.58, -0.01) ^c 3 µg/dL: -0.46 (-0.73, -0.19) ^c 4 µg/dL: -0.52 (-0.79, -0.24) ^c 5 µg/dL: -0.80 (-1.08, -0.51) ^c

Table 4-9 (Continued): Associations between blood or tooth Pb levels and measures of academic performance and achievement in children and young adults.

Study	Study Population and Methodological Details Organized by outcome then strength of study methodology, generalizability ^a	Blood (µg/dL) or Tooth Pb Metrics Analyzed	Measure of Academic Performance or Achievement Analyzed	Effect Estimate (95% CI) ^b
Min et al. (2009)	267 children followed from birth (1994-1996) to age 11 yr, Cleveland, OH Prospective. Recruitment at birth hospital. 86% African-American with high prevalence of prenatal drug and alcohol exposure. Moderate follow-up participation to age 4 yr, high retention to age 11 yr. Participants tended to be African-American and had married mothers. Linear regression model adjusted for HOME score, maternal birth vocabulary score, head circumference at birth, prenatal cocaine use (both outcomes), child sex, maternal prenatal alcohol use, current caregiver alcohol use. Also considered potential confounding by maternal education, Fe deficiency, maternal psychological distress, race.	Earlier childhood (Age 4 yr) Mean (SD): 7.0 (4.1) Interval analyzed: 3.0 (10th percentile)-10	Math	-0.45 (-0.84, -0.06)
			Reading	-0.60 (-1.0, -0.19)
			WJTA Age 11 yr	
Lanphear et al. (2000)	4,853 children ages 6-16 yr (born 1972-1988), U.S. NHANES III, 1988-1994 Cross-sectional. Large U.S. representative study of multiple risk factors and outcomes. Linear regression model adjusted for sex, race/ethnicity, poverty index ratio, reference adult education, serum ferritin and cotinine levels. Did not consider potential confounding by parental cognitive function or caregiving quality.	Concurrent Geometric mean (SD): 1.9 (7.0) 63.5% < 2.5 Detection limit = 0.5 Interval analyzed: 1-5	Math score	-0.70 (-1.0, -0.37)
			Reading score	-0.99 (-1.4, -0.62)
			WRAT-R Ages 6-16 yr	
Krieg et al. (2010)	766-780 children ages 12-16 yr (born 1975-1982), U.S. NHANES III, 1991-1994 Cross-sectional. Large U.S. representative study of multiple risk factors and outcomes. Log linear regression model adjusted for sex, caregiver education, family income, race/ethnicity, test language. Did not consider potential confounding by parental cognitive function or caregiving quality.	Concurrent Mean (5th-95th): 1.95 (1.63-2.27) Detection limit = 1 Interval analyzed: 1.7-2.2 = 10th-90th percentiles	Math score	-2.5 (-4.6, -0.38)
			Reading score	-2.9 (-4.4, -1.4)
			WRAT-R Ages 12-16 yr	

Table 4-9 (Continued): Associations between blood or tooth Pb levels and measures of academic performance and achievement in children and young adults.

Study	Study Population and Methodological Details Organized by outcome then strength of study methodology, generalizability ^a	Blood (µg/dL) or Tooth Pb Metrics Analyzed	Measure of Academic Performance or Achievement Analyzed	Effect Estimate (95% CI) ^b
Surkan et al. (2007)	389 children, ages 6-10 years, Boston, MA, Farmington, ME Cross-sectional. Recruitment from trial of amalgam fillings. High participation rate. Higher participation of white children in Maine. Analysis of covariance adjusted for caregiver IQ, child age, SES, race, IQ, birth weight. Also considered potential confounding by site, sex, birth order, caregiver education and marital status, parenting stress, and maternal utilization of prenatal and annual health care but not parental caregiving quality.	Concurrent		
		Group 1: 1-2	Reading score	Reference
		Group 2: 3-4		-0.93 (-3.8, 1.9) ^c
		Group 3: 5-10		-5.2 (-9.5, -0.95) ^c
		Group 1: 1-2	Math score	Reference
		Group 2: 3-4	WIAT	1.6 (-0.79, 4.0) ^c
Kordas et al. (2006)	293 children, ages 6-7 yr, Torreon, Mexico. Cross-sectional. Recruitment at schools. High participation rate. Residence near metal foundry. Linear regression model adjusted for child sex, age, school, birth order, hemoglobin, forgetting homework, household possessions and crowding, home ownership, maternal education, family structure, urinary arsenic, tester, school. Did not consider potential confounding by parental cognitive function or caregiving quality.	Group 3: 5-10	Ages 6-10 yr	-4.0 (-7.6, -0.43) ^c
		Mean (SD): 2.2 (1.6)		
		Concurrent	Math achievement test	-0.42 (-0.92, 0.08)
		Geometric mean (SD): 7.1 (1.7)	Ages 6-7 yr	
		Interval analyzed: 2-10		
Chiodo et al. (2007)	330-331 children, age 7 yr (born 1989-1991), Detroit, MI area. Cross-sectional. Recruitment at prenatal clinic. 100% African-American. High prevalence of prenatal drug exposure. High participation rate. Linear regression model adjusted for caregiver education, SES, HOME, maternal IQ and prenatal marijuana use, child sex (both outcomes). Caregiver concurrent psychological symptoms (Math), child age, maternal custody (Reading). Also considered potential confounding by prenatal cigarettes/day, alcohol use, cocaine use, # children in home, caretaker marital status, concurrent alcohol/week, current maternal cigarettes/day, and current marijuana use.	Concurrent	Math	-0.17 (-0.27, -0.07) ^d
		Mean (SD): 5.0 (3.0)	Reading	-0.06, p>0.05 ^e
		Interval analyzed: 2.1-8.7 = 10th-90th percentiles	Metropolitan Aptitude Test Age 7 yr	

Table 4-9 (Continued): Associations between blood or tooth Pb levels and measures of academic performance and achievement in children and young adults.

Study	Study Population and Methodological Details Organized by outcome then strength of study methodology, generalizability ^a	Blood (µg/dL) or Tooth Pb Metrics Analyzed	Measure of Academic Performance or Achievement Analyzed	Effect Estimate (95% CI) ^b
Chiodo et al. (2004)	237 children, age 7.5 yr, Detroit, MI area Cross-sectional. Recruitment at prenatal clinic. 100% African-American. High prevalence of prenatal alcohol and drug exposure. Moderate participation rate. Log linear regression model adjusted for SES, HOME, child sex, prenatal alcohol exposure, caregiver education and vocabulary. Also considered potential confounding by child age and life stress, maternal prenatal marijuana, smoking, or cocaine use, crowding, caregiver life stress, family functioning, # children <age 18 yr, parity.	Concurrent Mean (SD): 5.4 (3.3) Interval analyzed: 2.2-9.5 = 10th-90th percentiles	Arithmetic WISC-III Age 7.5 yr	-0.17, p>0.05 ^e
Studies of School Performance				
Fergusson et al. (1997)	881 children followed from birth to age 18 yr, Christchurch, New Zealand Prospective. Moderate follow-up participation, attrition did not affect results. Regression model adjusted for maternal age, punitiveness, standard of living, breastfeeding duration, parental conflict, grade, residence on busy roads. Also considered potential confounding by sex, ethnicity, maternal education, family size, HOME, SES, ethnicity, parental change, birth order, single parent.	Tooth Pb (age 6-8 yr) Mean (SD): 6.2 (3.7) µg/g See Ferguson et al. (1993)	Percent leaving school without school certificate Ages 16-18 yr	0-2 µg/g: 15.6 3-5 µg/g: 16.7 6-8 µg/g: 18.1 9-11 µg/g: 19.7 12+ µg/g: 24.1 p < 0.05
Needleman et al. (1990)	132 young adults followed from 1st/2nd grade (1975-1978) to age 18 yr, Chelsea, Somerville, MA Prospective. Recruitment at schools. Low follow-up participation. Participants had lower tooth Pb, higher parental education, SES, maternal IQ. Participation status did not influence results. Logistic regression adjusted for maternal age at birth, education, and IQ, family size, SES, sex, age at testing, birth order, alcohol use, mother and child left hospital together. Did not examine potential confounding by parental caregiving quality.	Tooth Pb (1st/2nd grade) distribution <10 ppm: 50% 10-19.9 ppm: 22.7% >20 ppm: 27.3%	Failure to graduate high school Highest grade achieved	7.4 (1.4, 41) ^d OR >20 ppm vs. <10 ppm -0.03 (-0.05, -0.01) per natural log increase in tooth Pb

Table 4-9 (Continued): Associations between blood or tooth Pb levels and measures of academic performance and achievement in children and young adults.

Study	Study Population and Methodological Details Organized by outcome then strength of study methodology, generalizability ^a	Blood (µg/dL) or Tooth Pb Metrics Analyzed	Measure of Academic Performance or Achievement Analyzed	Effect Estimate (95% CI) ^b
Study of teacher ratings of academic performance				
Leviton et al. (1993)	1,923 children followed from birth (1979-1980) to age 8 yr, Boston, MA area Prospective. Recruitment from birth hospital. Moderate participation at baseline and follow-up. Regression model adjusted for single parent family, gestational age, # children in home, maternal education, ethnicity, daycare in 1st 3 years. Also considered potential confounding by other unspecified factors.	Prenatal (cord) Mean: 6.8	Reading difficulty BTQ, Age 8 yr	RR (yes/no) per natural log increase
		Tooth Pb (Age 6 yr) Mean: 3.3 µg/g	Prenatal (cord)	Girls: 1.7 (0.9, 3.3) Boys: 1.3 (0.8, 2.2)
			Tooth Pb	Girls: 2.2 (1.1, 4.2) Boys: 1.2 (0.7, 2.0)

WRAT-R = Wide Range Achievement Test-Revised, WJTA = Woodcock Johnson-III Tests of Achievement, WIAT = Wechsler Individual Achievement Test, WISC = Wechsler Intelligence Scale for Children, BTQ = Boston Teacher Questionnaire.

^aResults are organized by method of outcome assessment. Within each category, results are grouped by strength of study design, representativeness of the population characteristics and blood Pb levels examined, and extent of consideration for potential confounding. There is not necessarily a continuum of decreasing strength across studies.

^bEffect estimates are standardized to a 1 µg/dL increase in blood Pb level in the interval from the 10th percentile of blood Pb level to the 90th percentile or 10 µg/dL, whichever is lower.

^cEffect estimates compare test performance of children in higher blood Pb groups to children in lowest blood Pb group.

^d95% CI was constructed using a standard error that was estimated from the reported p-value.

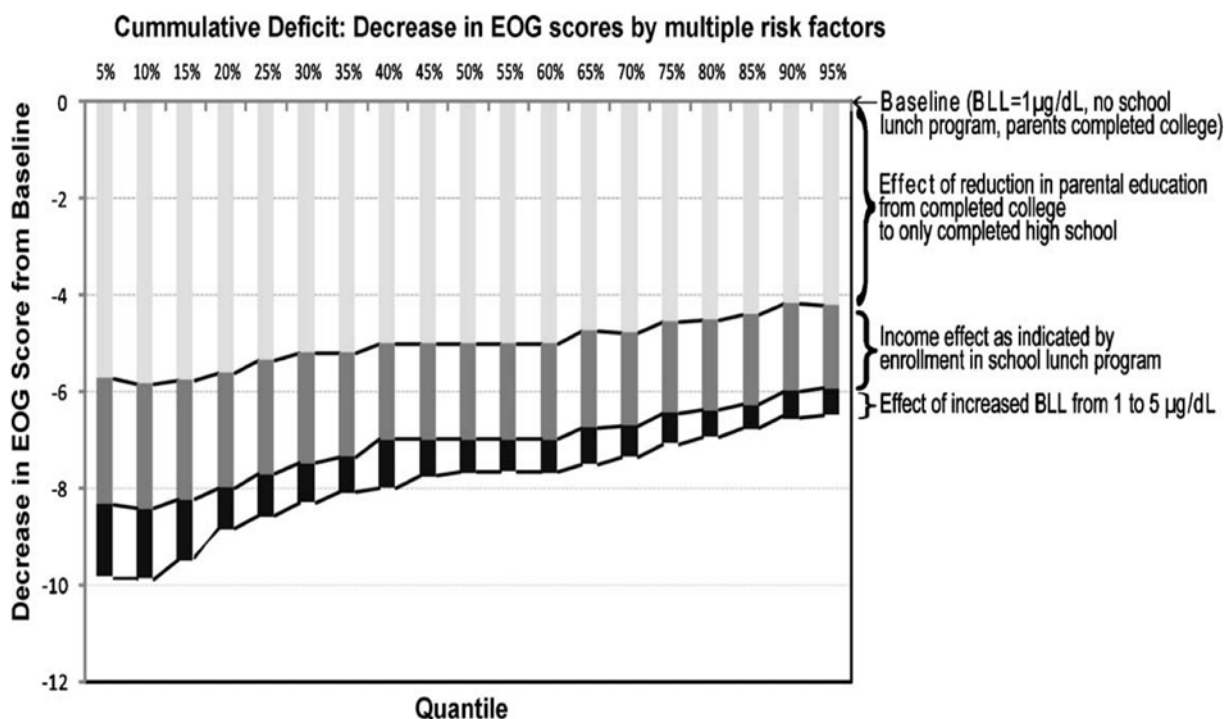
^eSufficient data were not available to calculate 95% CI.

Recent prospective studies found associations for early childhood blood Pb levels but did not have blood Pb measurements available at other time periods for comparison. The records-based analysis ([Miranda et al., 2009; 2007a](#)), recruitment at birth ([Min et al., 2009](#)), multi-factorial nature, or high participation rate of recent studies ([Chandramouli et al., 2009](#)) do not indicate a strong influence of selection bias. Miranda et al. ([2009; 2007a](#)) linked higher blood Pb levels measured at ages 0-5 years, as ascertained from a surveillance database, with lower end-of-grade (EOG) test scores in 8,603 fourth grade children in seven of the largest counties in North Carolina and then in 57,678 children in the entire state. A strength of the analyses was the availability of individual-level data on a large number of children representative of the North Carolina fourth grade population. The large numbers of children with blood Pb levels 2-5 µg/dL provided greater power to examine the effects of Pb in the lower range of blood Pb levels. In each analysis, children in the blood Pb level category of 2 µg/dL had lower reading and math fourth grade EOG scores ($p \leq 0.05$) compared with children in the blood Pb level category of 1 µg/dL (detection limit = 1 µg/dL). The reference blood Pb group comprised values below the detection limit and is subject to greater measurement error. This error likely is non-differential, which would make it more difficult to find an association if one exists. In linear models, the decrease in EOG score generally was monotonic across blood Pb groups. Because these children were born in the early- to mid-1990s and blood Pb levels were measured earlier in childhood, it is less likely that these children had much higher past Pb exposures and blood Pb levels that could have influenced the observed associations.

Due to the records-based study design, investigators had a smaller set of potential confounding factors available than those considered in the prospective studies described for other measures of cognitive function. Results were adjusted for sex, race, school-type, age of blood Pb measurement, parental education, participation in a free or reduced lunch program as a measure of SES, and in the analysis of seven North Carolina counties, school district and daily use of a computer as a measure of a stimulating home environment ([Miranda et al., 2007a](#)). While there may be no complete single measure of SES or parental caregiving quality, the covariates examined in these analyses are not as well characterized, and the results may be subject to some residual confounding.

In the statewide dataset, compared with children with an earlier (between ages 9 and 36 months) blood Pb level of 1 µg/dL, children with an earlier blood Pb level of 2 µg/dL had a 0.30-point lower (95% CI: -0.58, -0.01) fourth grade reading EOG score ([Miranda et al., 2009](#)). While the linear regression analyses assumed similar effects of Pb across the distribution, quantile regression indicated differential effects across the EOG distribution ([Figure 4-6](#)). Compared with linear regression, quantile regression is more robust in response to outliers and can identify whether effects differ at the top and bottom tails of

the outcome distribution rather than at the mean. Per unit increase in blood Pb level, there was a greater decrease in EOG in lower tail of the EOG distribution than in the middle or upper portions of the distribution (e.g., leftmost black bar versus rightmost black bar, [Figure 4-6](#)). For example, an increase in blood Pb level from 1 to 10 $\mu\text{g}/\text{dL}$ was associated with a 2.3-point decrease in EOG score in children in the 5th percentile of EOG and a 0.8-point decrease in children in the 95th percentile of EOG score. These findings indicated that children with the lowest EOG performance may be more affected by Pb exposure. Using quantile regression, Miranda et al. ([2009](#)) also showed that while indicators of lower SES such as lower parental education or enrollment in a school lunch program accounted for larger decrements in EOG score, an increase in blood Pb level was independently associated with EOG score decrements that were as large as 1 to 2 points.



Note: Baseline score calculated for a hypothetical referent individual with a blood Pb level of 1 $\mu\text{g}/\text{dL}$, having parents who completed college, and not enrolled in the school lunch program (i.e., model covariates = zero). Vertical bars indicate the decrease in EOG score associated with blood Pb level and covariates in various percentiles (quantiles) of EOG score (lowest to highest, left to right). An increase in early childhood blood Pb level was associated with a larger decrease in EOG score (larger black bars on left) among children in the 5th and 10th percentiles of EOG score than children in the 90th and 95th percentiles (smaller black bars on right).

Source: Reprinted with permission of Elsevier Science, Miranda et al. ([2009](#))

Figure 4-6 Greater reduction in End-of-Grade (EOG) scores for an increase in blood Pb level in lower percentiles of the test score distribution.

Similar to Miranda et al. (2009), Chandramouli et al. (2009) found associations between early childhood blood Pb levels (age 30 months) and later academic performance (Standard Assessment Test [SAT] at age 7 years). In this study of 488 children in the U.K., who had similar sociodemographic characteristics as those found in the U.K. census, a doubling of age 30 month blood Pb level was associated with a 0.3-point decline (95% CI: -0.5, -0.1) in SAT score. Results were adjusted for maternal education and smoking, home ownership, parental SES and several factors related to caregiving quality including home facilities score, family adversity index, and parenting attitudes. In analyses of blood Pb level groups, lower SAT scores were found in children with age 30 month blood Pb levels >5 µg/dL. Children with blood Pb levels 2-5 µg/dL did not have lower SAT scores than children with blood Pb levels 0-2 µg/dL.

Consistent with prospective studies, cross-sectional studies found associations between higher concurrent blood Pb level and lower scores on tests of math and reading, including large studies of children participating in NHANES. Cross-sectional studies considered potential confounding by SES and caregiver education, but few considered parental cognitive function or caregiving quality. Higher concurrent blood Pb level was associated with lower math and reading scores among 4,853 children ages 6-16 years and 766-780 children ages 12-16 years participating in NHANES (Krieg et al., 2010; Lanphear et al., 2000). The examination of multiple exposures and outcomes in NHANES increases confidence that associations are not unduly influenced by selection bias related specifically to Pb exposure. While mean concurrent blood Pb levels of these study populations were low, ~2 µg/dL, the influence of higher past Pb exposures on findings cannot be excluded. Consistent with studies of FSIQ, Lanphear et al. (2000) found a supralinear concentration-response relationship. A 1 µg/dL increase in concurrent blood Pb level was associated with a change in math score of -0.70 points (95% CI: -1.0, -0.37) among all subjects and -1.1 points (95% CI: -2.0, -0.12) among the 4,043 children ages 6-16 years with blood Pb levels <5 µg/dL. A supralinear relationship also was indicated in children age 7 years in Mexico living near a metal foundry by a larger blood Pb-associated decrement in math score among children with concurrent blood Pb levels <10 µg/dL (Kordas et al., 2006). These findings of nonlinearity based only on concurrent blood Pb without regard to early childhood blood Pb are less certain because the magnitude and timing of Pb exposures contributing to the associations are uncertain. In contrast with these findings, among children ages 6-10 years in New England, lower reading and math scores were related to higher concurrent blood Pb levels, i.e., blood Pb levels 5-10 µg/dL compared with blood Pb levels 0-2 µg/dL (Surkan et al., 2007).

Some studies found blood Pb-associated decreases math or reading test scores after greater consideration for potential confounding factors, including SES, maternal education, maternal intelligence, and parental caregiving quality. These associations were

found in populations with high prevalence of prenatal alcohol or drug exposure ([Min et al., 2009](#); [Chiodo et al., 2007](#); [2004](#)). These exposures, either in initial analyses or by inclusion in models with blood Pb, were not found to bias blood Pb results. Further, the results were consistent with those in more representative populations.

Prospective studies in a Boston, MA area cohort and New Zealand cohort found associations of tooth Pb levels measured at an earlier age (e.g., ages 6-8 years) with school performance ascertained at ages 18 years from school records ([Fergusson et al., 1997, 1993](#); [Needleman et al., 1990](#)), suggesting the effect of early exposure to Pb may be persistent. In the New Zealand cohort at ages 12-13 and 18 years, recruitment rate and follow-up participation were high, and model correction for nonrandom sample attrition produced robust results, indicating lack of undue selection bias ([Fergusson et al., 1997, 1993](#)). Further, associations observed at ages 12-13 years between higher tooth Pb level and lower teacher ratings of math, reading, and writing abilities ([Fergusson et al., 1993](#)), which are subject to greater measurement error, were supported by associations observed at age 18 years with more objective measures such as lower probability of leaving high school without a school certificate (i.e., completing high school) and lower scores on school exams ([Fergusson et al., 1997](#)). In this cohort, Pb-associated decrements in school performance were found with consideration for potential confounding by several factors including SES, parental education, HOME score, sex, ethnicity, number of school changes, perinatal history, breastfeeding, maternal age, and residence in weatherboard housing and near busy roads.

In one Boston-area cohort, those with age 6-7 year tooth Pb levels >20 ppm had greater risk (OR: 7.4 [95% CI: 1.4, 40.7]) of dropping out of high school at mean age 18 years compared with subjects with tooth Pb levels < 10 ppm ([Needleman et al., 1990](#)). The relatively small sample size (n = 132) and adjustment for several potential confounding factors, including maternal education, IQ, and age, SES, and subject alcohol use may have contributed to the imprecision of the effect estimate. Parental caregiving quality was not considered. Follow-up participation was biased to subjects with lower tooth Pb levels and higher SES. This selection bias likely did not produce a spurious association with Pb; however, the results may be less generalizable to the original study population. In another Boston-area cohort, higher tooth Pb level at age 6 years was associated with higher probability of having spelling and reading difficulties as rated by teachers in age 8-year girls but not boys ([Leviton et al., 1993](#)). This study had a large sample size (n = 1,923) and high follow-up participation but did not adjust for SES or parental caregiving quality. However, other unspecified potential confounding factors were considered.

4.3.2.6 Integrated Summary of Cognitive Function in Children

Results from recent epidemiologic and animal studies add to the strong evidence base reviewed in the 2006 Pb AQCD ([U.S. EPA, 2006b](#)) demonstrating that Pb exposure is associated with decrements in cognitive function in children, based strongly on associations observed with FSIQ, and also executive function and academic performance. Associations with performance on tests of learning and memory were less consistently found ([Table 4-5](#)). A large epidemiologic evidence base demonstrates associations of higher blood Pb level with lower FSIQ in children ages 3-17 years ([Figure 4-2](#) and [Table 4-3](#)), with smaller bodies of evidence indicating associations with lower scores on tests of executive function, and academic performance in children ages 4 to 18 years ([Table 4-8](#) and [Table 4-9](#)). There was no clear indication that blood Pb level was more strongly associated with performance in a particular domain of cognitive function. The Pb-associated decrements in cognitive function observed in children were strongly supported by observations in animals of decrements in learning, memory, and executive function with relevant dietary Pb exposures. In particular, coherence was found between observations of Pb-associated decrements in performance on spatial span tasks in children and on tasks in the Morris water maze in animals both of which test visual spatial memory. Coherence also was found for findings indicating Pb-associated decrements in performance on spatial working memory tasks in children and in animals with the radial arm maze ([Section 4.3.2.3](#)) and poorer cognitive flexibility in both humans and animals as assessed in tests of rule learning and reversal ([Section 4.3.2.4](#)). Additional biological plausibility for Pb-associated cognitive function decrements was provided by toxicological evidence for the effects of Pb on neurophysiological and neurochemical processes that mediate cognition.

Compelling epidemiologic evidence was described in the 2006 Pb AQCD ([U.S. EPA, 2006b](#)) for Pb-associated decrements in FSIQ (also see [Section 4.3.2.1](#) of this ISA). Across studies, FSIQ was measured with various instruments (i.e., WISC-R, WISC-III, WPPSI, Stanford-Binet) that were similar in their scoring scale and measurement error. Associations were found in most of the prospective studies, conducted in the U.S., Mexico, Europe, and Australia, in representative populations with moderate to high follow-up participation and without indication of selective participation among children with higher blood Pb levels and lower cognitive function ([Figure 4-2](#) and [Table 4-3](#)) that could produce spurious associations. The prospective studies found associations of blood Pb levels measured concurrently (ages 4-10 years) with FSIQ, measured earlier in life (i.e., prenatal cord or maternal, age 2 year or 4 year), or averaged over several years or a lifetime, characterizing the temporal sequence between Pb exposure and cognitive function decrements better than cross-sectional studies. Multiple testing was common; however, the consistent pattern of association observed across the ages of blood Pb level

and/or cognitive test examined in most previous and recent studies increases confidence that the evidence is not unduly biased by a higher probability of associations found by chance alone. Another strength of the prospective evidence was the consideration of several potential confounding factors. As indicated in [Table 4-3](#), results from most cohorts were adjusted for maternal IQ and education, child sex and birth weight, SES, and HOME score. Although not considered as frequently, some studies also indicated lack of confounding by parental smoking, birth order, and nutritional factors. The robustness of the blood Pb-FSIQ association in children was demonstrated in pooled analyses of seven prospective cohorts ([Lanphear et al., 2005](#); [Rothenberg and Rothenberg, 2005](#)) and multiple meta-analyses that combined results across various prospective and cross-sectional studies ([Pocock et al., 1994](#); [Schwartz, 1994](#); [Needleman and Gatsonis, 1990](#)), with Schwartz ([1994](#)) demonstrating the robustness of evidence to potential publication bias. While supporting the robustness of the blood Pb-FSIQ association, these analyses that combine data across different populations are subject to measurement error. Nondifferential error could result from the heterogeneity in the tests used to assess FSIQ and the ages of blood Pb and FSIQ examined. There also could be residual confounding due to heterogeneity among studies in the potential confounding factors examined and the method of assessment.

Among individual studies, a wide range of effect estimates was reported. Comparisons of effect estimates across studies are difficult because of the variability in population blood Pb distributions, lifestage or time period of blood Pb examined, type of model examined (linear versus nonlinear), and tests conducted. The pooled analysis of seven prospective cohorts demonstrated precision of effect estimates by finding a relatively narrow range of effect estimates, -2.4 to -2.9 points per log increase in blood Pb level, excluding one study at a time ([Lanphear et al., 2005](#)). In linear models, a greater decrease in FSIQ estimated for a 1 µg/dL increase in concurrent blood Pb for the 244 children who had peak blood Pb levels <10 µg/dL (-0.80 points [95% CI: -1.7, -0.14]) and the 103 children with peak blood Pb levels <7.5 µg/dL (-2.9 points [95% CI: -5.2, -0.71]). Among children with peak blood Pb levels <10 µg/dL and <7.5 µg/dL, the mean concurrent blood Pb levels were 4.3 µg/dL and 3.2 µg/dL, respectively ([Hornung, 2008](#)). Results from the Boston and Rochester cohorts can be considered particularly informative because they had lower blood Pb levels compared with other cohorts. Also, compared to the pooled analyses, there was homogeneity within each cohort in the tests used to assess FSIQ, the age of examination, and the methods used to measure potential confounding factors. Similarly large effects were estimated in the Boston and Rochester cohorts, which differed widely from each other in racial and SES distributions ([Canfield et al., 2003a](#); [Bellinger et al., 1992](#)). While the sample sizes were smaller ([Table 4-3](#)), these studies had at least as extensive consideration for potential confounding as other studies. Further, each study estimated larger effects for children whose peak blood Pb levels were

less than 10 µg/dL, -1.8 points (95% CI: -3.0, -0.60) per 1 µg/dL increase in concurrent blood Pb level in the Rochester cohort (n = 101, 59%) ([Canfield et al., 2003a](#)) and -1.6 points (95% CI: -2.9, -0.2) per 1 µg/dL in age 2-year blood Pb level in the Boston cohort (n = 48, 32%) ([Bellinger and Needleman, 2003](#)). These subsets of children had mean blood Pb levels of 3.3 (Rochester) and 3.8 µg/dL (Boston). Some recent cross-sectional studies estimated smaller effects but with examination of populations with higher concurrent blood Pb levels (means: 7, 8.1 µg/dL) using a linear model ([Kordas et al., 2011](#); [Min et al., 2009](#)). Other recent studies estimated similar effects as previous studies although the log-linear models make comparisons difficult. Among children ages 3-7 years in India, a 1 µg/dL increase in concurrent blood Pb level within the range from the 10th percentile (6.0) to 10 µg/dL was associated with a 0.54-point decrease (95% CI: -0.91, -0.17) in FSIQ ([Roy et al., 2011](#)). Among children ages 8-11 years in Korea, Kim et al. ([2009b](#)) found that a 1 µg/dL increase in concurrent blood Pb level in the 10th-90th percentile interval (0.9-2.8 µg/dL, detection limit = 0.06 µg/dL) was associated with decreases in FSIQ of 3.2 points (95% CI: -6.1, -0.24) and 2.4 points (-6.0, 1.1) in children with blood Mn levels >1.4 µg/dL and < 1.4 µg/dL, respectively. In this study, the potential influence of higher past Pb exposures cannot be excluded. Further, while these recent studies adjusted for parental education and SES, parental caregiving quality was not examined. The relatively low blood Pb levels in the Rochester and Boston cohorts, consideration of peak blood Pb levels, and the adjustment for several potential confounding factors indicate that their results may be more representative of the effects of Pb exposure.

Previous prospective studies, several of which contributed to the FSIQ evidence, provided key evidence for associations of blood or tooth Pb level with decrements in executive function and academic performance for the reasons described for FSIQ. Endpoints associated with blood or tooth Pb level included rule learning and reversal, reading and math skills assessed using neuropsychological tests and school performance assessed from testing or school records. Higher concurrent blood Pb level was associated with lower scores on tests of math and reading in large analyses of children participating in NHANES ([Krieg et al., 2010](#); [Lanphear et al., 2000](#)). Recent cross-sectional studies conducted in the U.S., Mexico, Europe, and Asia, also found associations of higher blood Pb level with poorer executive function or academic performance or achievement. The few recent prospective studies indicated associations between higher early childhood blood Pb level, ages 9-36 month and age 30 months, respectively, and poorer academic performance in fourth-grade children in North Carolina ([Miranda et al., 2009](#)) and in children ages 7-8 years in the U.K. ([Chandramouli et al., 2009](#)).

In most studies that provided unadjusted and adjusted effect estimates, blood Pb level was associated with smaller but statistically significant decrements in FSIQ after

adjusting for potential confounding factors ([Kim et al., 2009b](#); [Lanphear et al., 2005](#); [Canfield et al., 2003a](#)). The consideration for potential confounding varied among studies. Most studies adjusted for SES-related factors such as the Hollingshead Index, household income, and/or parental education. Several, in particular the prospective studies, adjusted for parental cognitive function and parental caregiving quality (mostly evaluated as HOME score). Overall, recent studies considered potential confounding by SES and parental IQ or education; few considered parental caregiving quality. Analyses of associations between potential confounding factors and blood Pb level and cognitive function indicated that the confounding factors may vary across populations and endpoints. In the Cleveland cohort, adjustment for HOME score attenuated the blood or tooth Pb level-FSIQ relationships ([Greene and Ernhart, 1993](#); [Greene et al., 1992](#); [Ernhart et al., 1988](#)). In the Rochester cohort, HOME score met the criteria for adjustment in models for FSIQ ([Canfield et al., 2003a](#)) but not all measures of memory and executive function ([Froehlich et al., 2007](#); [Canfield et al., 2004](#); [2003b](#)). Controlling for SES is difficult; it can be highly correlated with Pb exposure and there is no one measure that fully represents SES. Residual confounding also is likely by factors not considered or measured inadequately. The combination of evidence from prospective studies that considered several well-characterized potential confounding factors plus evidence that Pb exposure induces impairments in cognitive function in animals, in particular, spatial memory and executive function, which were associated with blood or tooth Pb levels in children increase confidence that the associations observed between blood and tooth Pb levels and cognitive function in children represent a relationship with Pb exposure.

With regard to important lifestages and durations of Pb exposure, toxicological evidence clearly demonstrates impaired learning and memory in animals exposed to Pb gestationally with or without additional postnatal exposure. The effect of early life Pb exposures is supported by evidence that processes such as neurogenesis and synaptic pruning are highly active during the first few years of life ([Rice and Barone, 2000](#); [Nolte, 1993](#)). However, evidence in a group of monkeys also indicates impaired learning with Pb exposure beginning later during the juvenile period, indicating that Pb exposure in gestation or in infancy is not necessary to induce impairments in cognitive function ([Rice, 1992b, 1990](#); [Rice and Gilbert, 1990b](#)). Epidemiologic studies also point to cognitive function decrements associated with blood Pb levels measured at various lifestages and time periods. Among studies of young children <3 years, several found stronger associations of MDI with prenatal (maternal or cord) blood Pb than with postnatal child blood Pb ([Hu et al., 2006](#); [Gomaa et al., 2002](#); [Bellinger et al., 1987](#); [Vimpani et al., 1985](#)). However, in older children, ages 4-17 years, in whom cognitive function is more stable and reliably measured, decrements in cognitive function were associated with prenatal and various postnatal (concurrent, early childhood, and multiple year or lifetime average) blood Pb metrics as well with tooth Pb levels. Evidence did not clearly identify

an individual critical lifestage, time period, or duration of Pb exposure in terms of risk of developing cognitive function decrements. Because bone Pb levels contribute to concurrent blood Pb levels in children and blood Pb levels generally show strong temporal correlation, associations with concurrent blood Pb levels may reflect an effect of past and recent Pb exposures.

Previous prospective studies found blood Pb-associated decrements in cognitive function in populations with mean blood Pb levels 5-10 µg/dL ([Table 4-3](#), [Table 4-5](#), [Table 4-8](#), and [Table 4-9](#)). In analyses restricted to children in the lower range of the blood Pb distribution (e.g., peak <10 µg/dL), associations with FSIQ at ages 6-10 years were found in groups of children with mean age 2 year or concurrent blood Pb levels 3-4 µg/dL with consideration of peak blood Pb levels ([Bellinger, 2008](#); [Canfield, 2008](#); [Hornung, 2008](#)). Additional evidence for effects at lower blood Pb levels was provided recently by Miranda et al. ([2009](#)), who found lower fourth grade EOG scores in children across North Carolina with age 9-36 month blood Pb level of 2 µg/dL compared to children with blood Pb level of 1 µg/dL. Other recent studies found associations in populations (ages 4-10 years) with mean blood Pb levels (mostly concurrent) 2-8 µg/dL ([Kordas et al., 2011](#); [Kim et al., 2009b](#); [Jusko et al., 2008](#); [Zailina et al., 2008](#); [Chiodo et al., 2007](#)) for FSIQ but not consistently for other measures of cognitive function ([Cho et al., 2010](#); [Chandramouli et al., 2009](#); [Surkan et al., 2007](#)). Kordas et al. ([2011](#)) examined concurrent blood Pb levels at a young age (e.g., age 4 years) with a mean of 8 µg/dL; other recent results for associations at lower blood Pb levels had greater uncertainty regarding influence of higher past Pb exposures or potential confounding. Recent animal studies added evidence for impaired learning, associative ability, and memory with blood Pb levels relevant to this ISA, 10-25 µg/dL ([Cory-Slechta et al., 2010](#); [Niu et al., 2009](#); [Stangle et al., 2007](#)). Some recent studies from the Cory-Slechta laboratory found greater learning impairments when Pb exposures were combined with stress, which potentially may be mediated via effects on corticosterone and dopamine ([Rossi-George et al., 2011](#); [Cory-Slechta et al., 2010](#); [Rossi-George et al., 2009](#); [Virgolini et al., 2008a](#); [2008b](#); [2005](#)).

Biological plausibility for the epidemiologic and animal evidence linking Pb exposure to decrements in cognitive function is provided by the well-characterized toxicological evidence for Pb exposure interfering with development of the brain and activity of neurochemical processes that mediate cognitive function ([Section 4.3.10](#)). Pb has been shown to increase the permeability of the blood-brain barrier and deposit in the CNS. Pb has been shown to impair neurogenesis, synaptic architecture, and neurite outgrowth. Cognitive function is mediated by the cortical and subcortical structures of the brain that integrate function in the hippocampus, prefrontal cortex, and nucleus accumbens using dopamine and glutamate as primary neurotransmitters. Experimental studies have shown

that Pb induces changes in dopamine and glutamate release in these regions and decreases LTP, which is a major cellular mechanism underlying synaptic plasticity and learning and memory.

4.3.2.7 Epidemiologic Studies of Cognitive Function in Adults

Adults without Occupational Pb Exposures

As described in the preceding section, Pb exposure that begins in gestation and lasts through the early postnatal period or lifetime exposure that begins in gestation or after infancy has been shown to induce learning impairments in adult animals. Less well characterized are learning impairments in adult animals due to adult-only Pb exposures. As reported in the 2006 Pb AQCD, epidemiologic studies have examined cognitive performance in adults without occupational Pb exposure primarily in association with concurrently measured blood and bone Pb levels and have found associations more with bone Pb levels than blood Pb levels ([U.S. EPA, 2006b](#)). Recent studies produced similar findings and provided new evidence from prospective analyses ([Table 4-10](#)).

Evidence was provided by large cohorts examining multiple exposures and outcomes, reducing the likelihood of selective participation of subjects specifically with higher Pb exposures and cognitive deficits. Most studies performed multiple tests of cognitive function. However, associations with bone Pb level were not isolated to a few tests. Several publications are available; however, many are variant analyses in the same population (e.g., Normative Aging Study [NAS], NHANES) and all are not considered independent assessments of the Pb-cognitive function relationship. Further, although some evidence is available from longitudinal cohorts, most analyses are cross-sectional examining the association between one measurement of cognitive function and a concurrent measure of blood or bone Pb level. Because temporality cannot be determined, causal inference regarding the effects of Pb exposure is limited. In analyses of bone Pb level, this limitation is mitigated somewhat because bone Pb level reflects several years of exposure. With blood or bone Pb level, it also is difficult to characterize the specific timing, duration, frequency, and level of Pb exposure that contributed to associations observed with cognitive function. This uncertainty may apply particularly to assessments of blood Pb levels, which in nonoccupationally-exposed adults, reflect both current exposures and cumulative Pb stores in bone that are mobilized during bone remodeling ([Sections 3.3](#) and [3.7.3](#)). Data on U.S. blood Pb levels from the 1960s-1980s indicate that for U.S. adults examined in the studies reviewed in this ISA, past blood Pb levels likely are within the range considered relevant for this ISA ([Sections 1.1](#) and [4.1](#)). Because of the greater difficulty in establishing the temporal sequence between Pb

exposure and cognitive function in cross-sectional studies, in the review of evidence, emphasis was placed on prospective analyses. Emphasis also was placed on studies that considered several potential confounding factors such as age, education, SES, smoking, and alcohol use. Even with the adjustment for age, there is the potential for residual confounding because of the strong correlation between increasing bone Pb levels and increasing age. However, the coherence with evidence for cognitive function decrements in animals induced by long-term Pb exposure provides support for epidemiologic results.

Evidence from Prospective Studies

Key evidence for the effects of Pb exposure on cognitive function of adults has been provided by recent prospective analyses of the large Baltimore Memory Study (BMS) and NAS. Strengths of these studies include the repeated assessment of Pb biomarker levels and cognitive performance, the high follow-up participation of subjects, and lack of selective attrition by Pb biomarker levels or demographic characteristics. In particular, the repeated assessments permitted examination of associations of bone Pb levels with changes in cognitive function over time, which better characterized the temporal sequence between Pb exposure and subsequent changes in cognitive function. The BMS and NAS had different strengths, including the sex and race of subjects and potential confounding factors considered. The BMS included men and women, ages 50-70 years, residing in Baltimore, MD. A total of 1,140 out of 2,351 (48.5%) subjects participated from neighborhoods that represented a diversity of race and SES. This study was unique in that it included a large proportion of African Americans (n = 395). In comparison, the NAS involved only men (original n = 2,280) residing in the Boston, MA area. Subjects primarily were white and at enrollment were ages 21 to 80 years and had no current or past chronic medical conditions. Both studies adjusted for age and education. The BMS additionally adjusted for household wealth, and the NAS additionally adjusted for intake of alcohol and smoking. Results from both of these cohorts with different demographics and analytical methods indicated Pb-associated cognitive function decrements.

In the BMS, longitudinal analyses involved repeat cognitive testing at 14-month intervals. Most subjects completed follow-up; 91% at the second round of testing and 83% at the third round ([Bandeem-Roche et al., 2009](#)). An interquartile range higher baseline tibia Pb level (12.7 µg/g) was associated with a 0.019 unit per year decrease (95% CI: -0.031, -0.007) in eye-hand coordination z-score, with adjustment for age, sex, race, SES, and interviewer, with a larger decrease estimated for African Americans than for whites ([Table 4-10](#)). Results were not homogeneous across the various tests performed. Tibia Pb levels were more weakly associated with time-related decreases in language, processing speed, and executive function; however, most effect estimates were

negative in direction. Further, for language and executive function, tibia Pb level was associated with greater decreases in scores among whites than African Americans

Similar to the BMS, among NAS men, higher baseline tibia Pb levels were associated with decreases in cognitive performance over time in longitudinal analyses with repeated measures of cognitive function plus a bone Pb-time interaction term in order to estimate the association between baseline bone Pb level and decline in cognitive test score over time ([Weisskopf et al., 2007b](#)). This NAS analysis expanded the evidence base by also finding associations with patella Pb levels. Two measurements of cognitive function, collected approximately 3.5 years apart were available for 60-70% of participants. Both tibia and patella Pb levels were associated with decrements in executive function, short-term memory, and visuospatial skills (as indicated by increased response latency on a pattern comparison test). The strongest effect was estimated for the latter. Weisskopf et al. ([2007b](#)) also found a nonlinear association with patella Pb, with Pb-associated latency times becoming worse over time (i.e., larger values indicating slower response time) up to approximately 60 µg/g patella Pb then leveling off at higher levels ([Figure 4-7](#)). A 20 µg/g difference in patella Pb level was associated with an increase in latency of 0.073 ms (95% CI: 0.04, 0.12) among all men and a 0.15 ms increase among men with patella Pb level <60 µg/g. Both patella and tibia Pb were associated with fewer errors on the pattern comparison test. The authors proposed that this association in the unexpected direction may be related to slowing reaction time to improve accuracy. When the nine men with the highest bone Pb levels were removed, the association with fewer errors was no longer statistically significant. However, the authors did not indicate whether the point estimate changed.

Table 4-10 Associations of blood and bone Pb levels with cognitive function in adults.

Study Population and Methodological Details		Subgroup (where examined)	Blood Pb Effect Estimate	Bone Pb Effect Estimate
Prospective analyses presented 1st, then cross-sectional analyses. Within categories, studies are grouped by strength of methodology, generalizability ^a			(95% CI)	(95% CI)
Prospective Analyses				
Bandeem-Roche et al. (2009) 965, ages 50-70 yr at baseline, BMS, Baltimore, MD Large sample of men and women of various races/ethnicities with repeated measures of cognitive function and tibia Pb. High follow-up participation over 28 mo. Marginal linear regression models adjusted for age, sex, household wealth, education, race/ethnicity, interviewer. Did not consider potential confounding by history of smoking or alcohol use. Tibia Pb Mean (SD): 19 (12.7) µg/g	Longitudinal associations		NOT EXAMINED	Change in Z-score per IQR increase:
	Eye/hand coordination - Purdue pegboard, trail-making test	African- American		-0.032 (-0.052, -0.012)/yr
		White		-0.009 (-0.024, 0.006)/yr
	Cross-sectional associations			
	Verbal memory/learning – Rey auditory verbal learning test	African-American		0.006 (-0.09, 0.10)
		White		-0.076 (-0.153, 0.001)
	Language – Boston naming test	African-American		0.065 (-0.010, 0.139)
		White		-0.024 (-0.076, 0.028)

Table 4-10 (Continued): Associations of blood and bone Pb levels with cognitive function in adults.

Study Population and Methodological Details		Subgroup (where examined)	Blood Pb Effect Estimate (95% CI)	Bone Pb Effect Estimate (95% CI)
Prospective analyses presented 1st, then cross-sectional analyses. Within categories, studies are grouped by strength of methodology, generalizability ^a			Cognitive Test	
Weisskopf et al. (2007b) 401-750 males, mean age 68.7 yr at baseline, NAS, Boston, MA area. Large sample, only men, primarily white. Repeated measures of cognitive function and tibia Pb. Moderate follow-up participation over 3.5 yr. Linear repeated measures analysis adjusted for age, age ² , education, smoking status, current alcohol intake, yr between bone Pb measurement and first cognitive test, yr between cognitive tests. Also evaluated language, computer experience, physical activity. Did not consider potential confounding by other SES variables. Mean (IQR) Tibia Pb: 20 (15) µg/g Patella Pb: 25 (20) µg/g	Longitudinal associations		NOT EXAMINED	Change in test score over 3.5 yr per IQR increase:
	Visuospatial skills - pattern comparison latency (negative = poorer performance), NES2			Tibia: -0.079 (-0.12, -0.04) Patella: -0.073 (-0.12, -0.04)
	Executive function - verbal fluency, WISC-R			Tibia: -0.04 (-0.16, 0.08) Patella: -0.086 (-0.20, 0.03)
	Short-term memory - # words recalled, CERAD			Tibia: -0.028 (-0.12, 0.06) Patella: -0.081 (-0.17, 0.005)
	Cross-sectional associations			
	Visuospatial skills - pattern comparison latency (negative = poorer performance), NES2			Tibia: 0.03 (-0.11, 0.17) Patella: 0.02 (-0.11, 0.14)
	Executive function - verbal fluency, WISC-R			Tibia: -0.27 (-0.70, 0.16) Patella: -0.22 (-0.62, 0.17)
	Short-term memory – # words recalled, CERAD			Tibia: 0.12 (-0.20, 0.32) Patella: 0.012 (-0.18, 0.41)
Wang et al. (2007a) 358 males, median age: 67 yr, NAS, Boston, MA area Same cohort as above. Subset representative of full cohort. Linear regression adjusted for age, years of education, smoking status, pack-years smoking, nondrinker, grams/day alcohol consumption, English as first language, computer experience, diabetes. Did not consider potential confounding by other SES variables. Tibia Pb Median (IQR): 19 (15) µg/g	Mini Mental State Exam Score	HFE wildtype	NOT EXAMINED	Change in test score per IQR increase:
		1 HFE variant		-0.02 (-0.10, 0.07)/yr
		2 HFE variants		-0.14 (-0.33, 0.04)/yr
				-0.63 (-1.04, -0.21)/yr

Table 4-10 (Continued): Associations of blood and bone Pb levels with cognitive function in adults.

Study Population and Methodological Details			Blood Pb Effect Estimate (95% CI)	Bone Pb Effect Estimate (95% CI)
Prospective analyses presented 1st, then cross-sectional analyses. Within categories, studies are grouped by strength of methodology, generalizability ^a	Cognitive Test	Subgroup (where examined)		
Cross-sectional Analyses				
Shih et al. (2006)			Z-score per 1 µg/dL increase:	Z-score per 1 µg/g increase:
985 adults, mean age: 59 yr, BMS, Baltimore, MD				
Large sample of men and women of various races/ethnicities. Subjects with tibia Pb measured were more educated and white. Compared blood/bone associations.	Language - Boston naming test	Model A	-0.006 (-0.03, 0.017)	-0.008 (-0.01, -0.004)
		Model B	-0.002 (-0.02, 0.016)	0.0006 (-0.003, 0.004)
Linear regression adjusted for:	Eye-hand coordination - Purdue Pegboard, trail making	Model A	-0.011 (-0.03, 0.01)	-0.008 (-0.01, -0.004)
Model A: age, sex, technician, presence of APOE-ε4 allele		Model B	-0.008 (-0.03, 0.01)	-0.003 (-0.006, 0.001)
Model B: Model I, years of education, race/ethnicity, wealth	Executive functioning – Purdue Pegboard, Stroop, trail making test	Model A	-0.014 (-0.03, 0.005)	-0.008 (-0.01, -0.004)
		Model B	-0.010 (-0.03, 0.007)	-0.001 (-0.005, 0.002)
Did not consider potential confounding by history of smoking or alcohol use.	Visuoconstruction – Rey complex figure copy	Model A	-0.019 (-0.05, 0.008)	-0.012 (-0.02, -0.007)
Mean (SD)		Model B	-0.014 (-0.04, 0.01)	-0.004 (-0.009, 0.0003)
Concurrent blood Pb: 3.5 (2.2) µg/dL				
Tibia Pb: 18.7 (11.2) µg/g				
Glass et al. (2009)			NOT EXAMINED	Standardized score per 1 µg/g increase:
1,001 adults, mean age 59 yr, BMS, Baltimore, MD				
Same cohort as above. High participation rate: 91%.	Language - Boston naming test	2nd tertile NPH		0.001 (-0.008, 0.009) ^b
		3rd tertile NPH		-0.009 (-0.017, -0.0001) ^b
Multilevel hierarchical regression model adjusted for age, sex, race/ethnicity, education, testing technician, time of day tested. Investigator assessed NPH. # of NPHs increase across tertiles.	Eye-hand coordination–Purdue Pegboard, trail making test	2nd tertile NPH		-0.004 (-0.012, 0.005) ^b
Did not consider potential confounding by history of smoking or alcohol use.		3rd tertile NPH		-0.006 (-0.015, 0.002) ^b
Tibia Pb Mean (SD): 18.8 (11.1) µg/g	Executive functioning–Purdue Pegboard, Stroop, trail making test	2nd tertile NPH		-0.002 (-0.010, 0.006) ^b
		3rd tertile NPH		-0.010 (-0.018, -0.002) ^b
	Visuoconstruction – Rey complex figure copy	2nd tertile NPH		-0.003 (-0.014, 0.008) ^b
		3rd tertile NPH		-0.006 (-0.017, 0.005) ^b

Table 4-10 (Continued): Associations of blood and bone Pb levels with cognitive function in adults.

Study Population and Methodological Details		Cognitive Test	Subgroup (where examined)	Blood Pb Effect Estimate (95% CI)	Bone Pb Effect Estimate (95% CI)
Prospective analyses presented 1st, then cross-sectional analyses. Within categories, studies are grouped by strength of methodology, generalizability ^a					
Weuve et al. (2006)		Mini Mental State Exam Score		Test score per IQR increase:	Test score per IQR increase:
720-915 males, ages ≥ 45 yr, NAS, Boston, MA area			ALAD1-1	-0.04 (-0.16, 0.07)	Tibia: -0.05 (-0.21, 0.12)
Large sample, only men, primarily white. Moderate follow-up participation. Compared blood/bone associations. Linear mixed effects regression adjusted for age, education, computer experience, time between Pb and cognitive assessment, smoking status, grams/day alcohol consumption, calorie adjusted calcium intake, regular energy expenditure on leisure time physical activity, diabetes. Additional adjustment for dietary factors. n = 766 ALAD1-1, n = 149 ALAD-2			ALAD-2 carrier	-0.26 (-0.54, 0.01)	Tibia: -0.16 (-0.58, 0.27)
			ALAD1-1		Patella: -0.07 (-0.23, 0.09)
			ALAD-2 carrier		Patella: -0.26 (-0.64, 0.12)
Median (IQR)					
Concurrent blood Pb: 5.2 (3) µg/dL					
Tibia Pb: 19 (15) µg/g					
Patella Pb: 27 (21) µg/g					

Table 4-10 (Continued): Associations of blood and bone Pb levels with cognitive function in adults.

Study Population and Methodological Details		Cognitive Test	Subgroup (where examined)	Blood Pb Effect Estimate (95% CI)	Bone Pb Effect Estimate (95% CI)
Prospective analyses presented 1st, then cross-sectional analyses. Within categories, studies are grouped by strength of methodology, generalizability ^a					
Rajan et al. (2008)				Z-score*ALAD-2 per IQR increase:	Z-score*ALAD-2 per IQR increase:
486-959 males, ages ≥ 49 yr, NAS, Boston, MA area		Visuospatial - constructional Praxis, CERAD		-0.05 (-0.23, 0.13) ^c	Tibia: -0.25 (-0.49, -0.02) ^c Patella: 0.02 (-0.19, 0.23) ^c
Same cohort as above. Compared blood/bone associations. Linear regression adjusted for blood Pb main effect, ALAD genotype, age at cognitive test, education, grams/day alcohol consumption, pack-years smoking, English as first language. Also considered smoking status, income, physical activity, diabetes, coronary heart disease. n = 818 ALAD1-1, n = 164 ALAD-2		Executive function - verbal fluency, CERAD		-0.03 (-0.22, 0.16) ^c	Tibia: -0.11 (-0.34, 0.13) ^c Patella: -0.03 (-0.24, 0.19) ^c
Concurrent blood Pb Mean (SD)		Verbal memory - word list memory, CERAD		0.003 (-0.18, 0.19) ^c	Tibia: 0.08 (-0.15, 0.31) ^c Patella: 0.14 (-0.07, 0.34) ^c
ALAD1-1: 5.3 (2.9) µg/dL		Perceptual speed - mean response latency (negative = poorer performance)		-0.18 (-0.42, 0.06) ^c	Tibia: -0.25 (-0.59, 0.08) ^c Patella: -0.16 (-0.44, 0.12) ^c
ALAD-2 carriers: 4.8 (2.7) µg/dL		continuous performance, NES			
Tibia Mean (SD)					
ALAD1-1: 21.9 (13.8) µg/g					
ALAD-2 carriers: 21.2 (11.6) µg/g					
Patella Mean (SD)					
ALAD1-1: 29.3 (19.1) µg/g					
ALAD-2 carriers: 27.9 (17.3) µg/g					

Table 4-10 (Continued): Associations of blood and bone Pb levels with cognitive function in adults.

Study Population and Methodological Details			Blood Pb Effect Estimate (95% CI)	Bone Pb Effect Estimate (95% CI)
Prospective analyses presented 1st, then cross-sectional analyses. Within categories, studies are grouped by strength of methodology, generalizability ^a				
Cognitive Test	Subgroup (where examined)			
Weuve et al. (2009) 587 females, ages 47-74 yr, Nurses' Health Study, Boston, MA area Large sample of only females, primarily white. Compared blood/bone associations. Generalized estimating equations adjusted for age and age² at Pb assessment, age at cognitive assessment, education, husband's education, alcoholic drinks/week, smoking status, physical activity, use of aspirin, ibuprofen, Vitamin E supplements, menopausal status, and postmenopausal hormone use. Additional adjustment for nutritional factors, medication use, vascular and mental health. Assessed outcomes over telephone but a mean 5 years after Pb measured. Mean (SD) Concurrent blood Pb: 2.9 (1.9) µg/dL Tibia Pb: 10.5 (9.7) µg/g Patella Pb: 12.6 (11.6) µg/g			Z-score per SD increase:	Z-score per SD increase:
	Orientation, registration, immediate verbal memory with TICS. Immediate and delayed paragraph recall, category fluency, digit span backwards (working memory, attention) with EBMT			
	Composite Z-score		-0.015 (-0.069, 0.039)	Tibia: -0.040 (-0.09, 0.004) Patella: -0.012 (-0.06, 0.03)
	Composite except letter fluency		0.016 (-0.071, 0.039)	Tibia: -0.05 (-0.10, -0.003) Patella: -0.033 (-0.08, 0.014)
Krieg and Butler (2009) 2,823 adults, ages 20-59 yr, Large U.S. representative NHANES III (1991-1994). Log-linear regression model adjusted for age, sex, education, family income, race-ethnicity, computer or video-game familiarity, alcohol use within the last 3 h, test language, sampling unit and stratum. Did not consider potential confounding by smoking. Concurrent Blood mean (SD): 2.88 (6.91) µg/dL			Test score per log increase:	NOT EXAMINED
	Symbol Digit Substitution (mean total latency, sec)	Ages 20-39 yr	-0.41 (-1.7, 0.83) ^d	
		Ages 40-59 yr	-1.5 (-3.0, 0.05) ^d	
	Serial digit learning total score, NES	Ages 20-39 yr	0.32 (-0.60, 1.3)	
		Ages 40-59 yr	-1.1 (-2.6, 0.43)	

Table 4-10 (Continued): Associations of blood and bone Pb levels with cognitive function in adults.

Study Population and Methodological Details		Cognitive Test	Subgroup (where examined)	Blood Pb Effect Estimate (95% CI)	Bone Pb Effect Estimate (95% CI)	
Prospective analyses presented 1st, then cross-sectional analyses. Within categories, studies are grouped by strength of methodology, generalizability ^a						
Krieg et al. (2009)			ALAD rs1800435 genotype	Test score per log increase	NOT EXAMINED	
1,852-1,952 adults ages 20-59 yr, 1,551-1,740 adults ages ≥ 60 yr. Large U.S. representative NHANES III (1991-1994).		Symbol Digit Substitution (mean total latency)	Ages 20-59 yr			
Log linear regression model adjusted for sex, age, education, family income, race-ethnicity, computer or video game familiarity, alcohol use in the last 3 h, test language (ages 20-59 yr) and sex, age, education, family income, race-ethnicity, test language (ages ≥ 60 yr), sampling unit and stratum. Did not consider potential confounding by smoking.			CC/CG	-2.7 (-5.7, 0.30) ^d		
			GG	-0.68 (-1.8, 0.48) ^d		
		Serial digit learning total score		Ages 20-59 yr		
Concurrent Blood Pb Mean (SD)		Serial digit learning total score	CC/CG	-1.1 (-4.0, 1.8)		
			GG	0.05 (-1.3, 1.4)		
			Word recall, number correct		Ages ≥ 60 yr	
Age 20-59 yr: 2.85 (7.31) µg/dL		Word recall, number correct	CC/CG	0.02 (-0.33, 0.37)		
Age ≥ 60 yr: 4.02 (3.39) µg/dL			GG	-0.06 (-0.23, 0.10)		
			Story recall, number correct, Neurobehavioral Evaluation System		Ages ≥ 60 yr	
				CC/CG	-0.88 (-2.0, 0.26)	
GG	0.16 (-0.19, 0.52)					

Table 4-10 (Continued): Associations of blood and bone Pb levels with cognitive function in adults.

Study Population and Methodological Details		Cognitive Test	Subgroup (where examined)	Blood Pb Effect Estimate (95% CI)	Bone Pb Effect Estimate (95% CI)
Prospective analyses presented 1st, then cross-sectional analyses. Within categories, studies are grouped by strength of methodology, generalizability ^a					
Krieg et al. (2010)			By VDR variant	Test score per log increase:	NOT EXAMINED
1,852-1,952 adults ages 20-59 yr, 1,551-1,740 adults, ages ≥ 60 yr. Large U.S. representative NHANES III (1991-1994).		Symbol Digit Substitution (mean total latency, sec)	Ages 20-59 yr		
Log linear regression model adjusted for sex, age, education, family income, race-ethnicity, computer or video game familiarity, alcohol use in the last 3h, test language, sampling unit, stratum (ages 20-59 yr) and sex, age, education, family income, race-ethnicity, test language, sampling unit, and stratum (ages ≥ 60 yr). Did not consider potential confounding by smoking.			CC haplotype	-20 (-44, 4.0) ^d	
			CT haplotype	0.73 (-1.4, 2.9) ^d	
			TC haplotype	-2.6 (-5.3, 0.07) ^d	
			TT haplotype	-3.6 (-7.2, 0.05) ^d	
		Serial digit learning total score		Ages 20-59 yr	
Concurrent blood Pb Mean (SD) Age 20-59 yr: 2.85 (7.31) µg/dL Age ≥ 60 yr: 4.02 (3.39) µg/dL		CC haplotype	8.0 (0.61, 15.4)		
		CT haplotype	1.0 (-0.89, 2.9)		
		TC haplotype	-1.4 (-3.1, 0.29)		
		TT haplotype	-0.14 (-2.8, 2.5)		
		Word recall, number correct		Ages ≥ 60 yr	
Story recall, number correct Neurobehavioral Evaluation System		CC haplotype	-0.65 (-1.5, 0.25)		
		CT haplotype	-0.08 (-0.34, 0.19)		
		TC haplotype	-0.03 (-0.40, 0.33)		
		TT haplotype	-0.08 (-0.76, 0.60)		
		CC haplotype	0.29 (-3.3, 3.9)		
		CT haplotype	0.01 (-0.38, 0.40)		
		TC haplotype	0.07 (-0.64, 0.78)		
		TT haplotype	-0.22 (-0.86, 0.43)		

Table 4-10 (Continued): Associations of blood and bone Pb levels with cognitive function in adults.

Study Population and Methodological Details		Subgroup (where examined)	Blood Pb Effect Estimate (95% CI)	Bone Pb Effect Estimate (95% CI)
Prospective analyses presented 1st, then cross-sectional analyses. Within categories, studies are grouped by strength of methodology, generalizability ^a	Cognitive Test			
Van Wijngaarden et al. (2009) 47 adults, ages 55-67 yr, Rochester, NY Small sample size. Linear regression adjusted for age, smoking status, educational level, history of hypertension. Did not consider potential confounding by other SES variables. Excluded subjects with BMI >32 kg/m ² . Mean (SD) Tibia Pb: 2.0 (5.2) µg/g Calcaneus Pb: 6.1 (8.5) µg/g	Delayed matching to sample, % correct CANTAB		NOT EXAMINED	Calcaneus Lowest tertile: 85.40 ^e Medium tertile: 85.34 Highest Tertile: 80.83, p=0.07 Tibia Lowest tertile: 83.04 ^e Medium tertile: 85.91 Highest tertile: 81.85, p=0.32
	Paired Associate Learning, total trials adjusted (increase = poorer performance) CANTAB			Calcaneus Lowest tertile: 2.61 ^e Medium tertile: 2.68 Highest tertile: 2.74, p = 0.25 Tibia Lowest tertile: 2.69 ^e Medium tertile: 2.64 Highest tertile: 2.72, p = 0.70

Table 4-10 (Continued): Associations of blood and bone Pb levels with cognitive function in adults.

Study Population and Methodological Details		Subgroup (where examined)	Blood Pb Effect Estimate	Bone Pb Effect Estimate
Prospective analyses presented 1st, then cross-sectional analyses. Within categories, studies are grouped by strength of methodology, generalizability ^a			(95% CI)	(95% CI)
Gao et al. (2008)	Composite cognitive Z-score		Z-score per 1 µg/L plasma Pb increase:	NOT EXAMINED
188 adults, mean age 69.2 yr, Rural Sichuan and Shandong Provinces, China.	Word list learning, word recall (CERAD), CSID, IU story recall, Animal naming fluency test, IU token test of language and working memory		-0.009 (-0.025, 0.007)	
Small sample size. Subset was younger, had more education, and higher BMI than full cohort.				
Separate ANCOVAs adjusted for age, sex, education, BMI, or APOE ε4. History of smoking and alcohol consumption not associated with cognitive score.				
Concurrent plasma Pb Mean (SD): 0.39 (0.63) µg/dL				

Note: Effect estimates in bold indicate the stronger association between blood Pb and bone Pb level. IQR = Interquartile range, BMS = Baltimore Memory Study, NAS = Normative Aging Study, NES2 = Neurobehavioral Evaluation System 2, WISC-R = Wechsler Adult Intelligence Scale-Revised, CERAD = Consortium to Establish Registry for Alzheimer's disease, HFE = Human Hemochromatosis protein, NPH = Neighborhood Psychosocial Hazard, TICS = Telephone Interview for Cognitive Status, EBMT = East Boston Memory Test, CANTAB = Cambridge Neuropsychological Test Automated Battery, CSID = Community Screening Instrument for Dementia, IU = Indiana University.

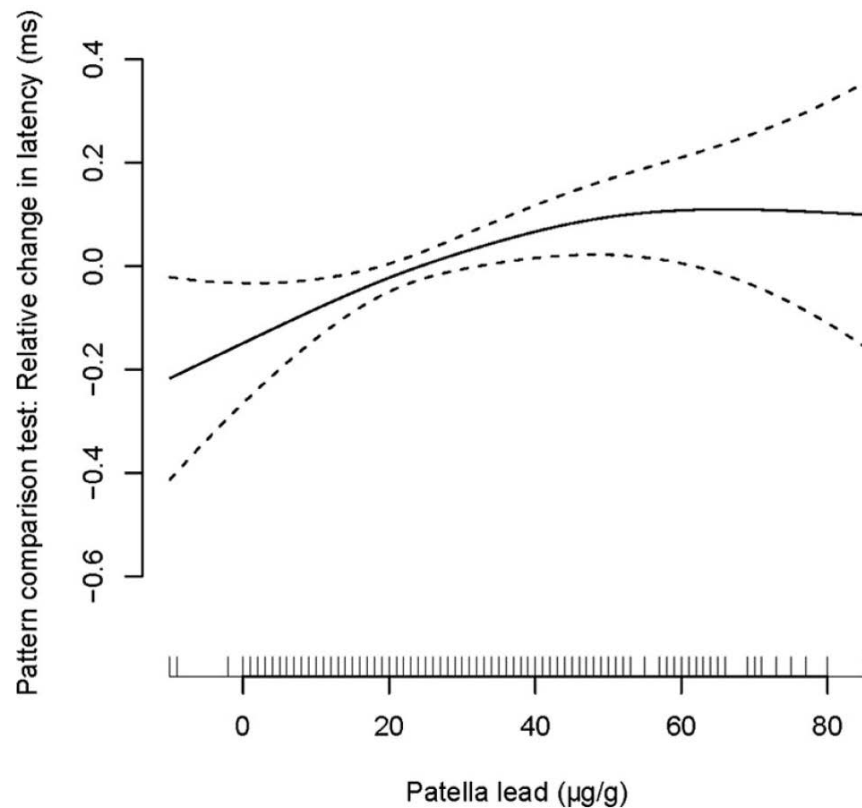
^aStudies are presented first for prospective analyses then for cross sectional analyses. Within categories, results are grouped by representativeness of the population characteristics and blood Pb levels examined and extent of consideration for potential confounding. There is not necessarily a continuum of decreasing strength across studies.

^bEffect estimates are for interactions between Pb and category of NPH, with the lowest tertile of NPH (fewer NPH) serving as the reference group.

^cEffect estimates are for interactions between Pb and ALAD genotype.

^dThe directions of effect estimates were changed to indicate a negative slope as a decrease in cognitive performance.

^eResults refer to mean cognitive test scores among tertiles of bone Pb. Tertile concentrations not reported.



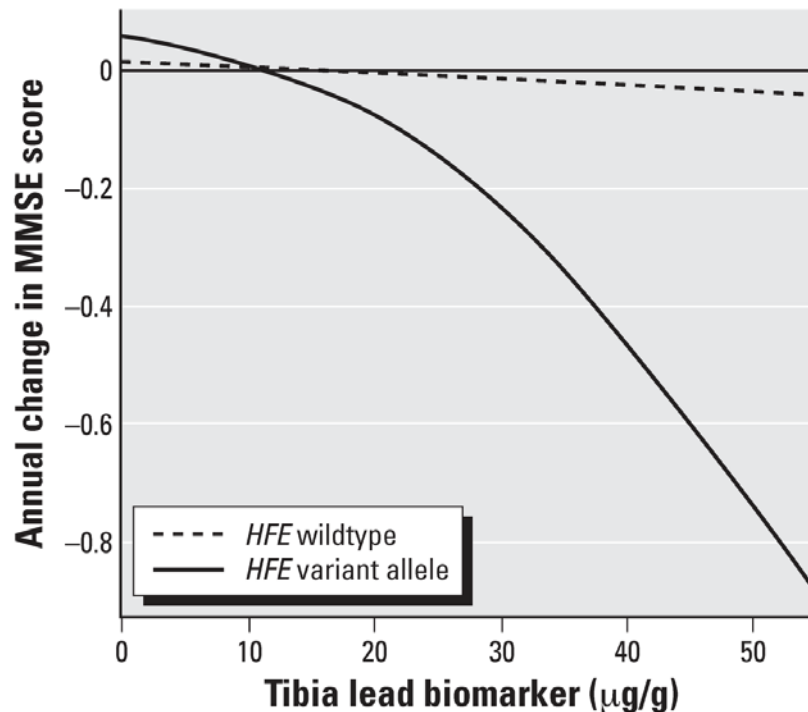
Note: Models are adjusted for age, age squared, education, smoking, alcohol intake, years between bone Pb measurement and first cognitive test, and years between the cognitive tests. The 9 subjects with the highest patella Pb levels ($>89 \mu\text{g/g}$ bone mineral) were removed. The estimated concentration-response is indicated by the solid line and the 95% confidence interval by the dashed lines. The patella Pb level-associated increase in response latency (reference = 0 at mean of patella Pb level) is larger among men with patella Pb levels $<60 \mu\text{g/g}$. Patella Pb levels of individual subjects are indicated by short vertical lines on the abscissa.

Source: Reprinted with permission of Williams & Wilkins, Weisskopf et al. (2007b).

Figure 4-7 Nonlinear association between patella Pb level and the relative change over 3.5 years in response latency on the pattern comparison test in men from the Normative Aging Study.

Longitudinal analysis of the NAS cohort also indicated that hemochromatosis (HFE) gene variants modified the blood Pb-cognition association (Wang et al., 2007a). In models adjusted for age, years of education, smoking status, pack-years smoking, nondrinker, grams/day alcohol consumption, English as first language, computer experience, and diabetes, an interquartile range higher tibia Pb level ($15 \mu\text{g/g}$) was associated with a 0.22 point steeper annual decline (95% CI: -0.39, -0.05) in Mini-Mental State Examination score (MMSE, which assesses cognitive impairment in a number of domains) among the 130 (36%) men with either the H63D or C282Y variant. The

association was found to be nonlinear, with larger Pb-associated declines observed at higher tibia Pb levels (Figure 4-8, solid curved line). The change in MMSE score associated with a 15 µg/g higher tibia Pb level was comparable to that found between NAS men who were 4 years apart in age. Tibia Pb level was not associated with a decline in MMSE score in men with the HFE wildtype genotype (Figure 4-8, dashed line). Bone Pb levels did not differ widely by HFE variant. HFE variants, H63D and C282Y, are associated with hemochromatosis, a disease characterized by higher Fe body burden. Iron metabolism has been hypothesized to affect neurodegenerative diseases, which may explain the observed effect modification. However, firm conclusions regarding effect modification of the bone Pb-cognition relationship by HFE variants are not warranted.



Note: The lines indicate curvilinear trends estimated from the penalized spline method. Among hemochromatosis (HFE) wild-types, the association between tibia Pb and annual cognitive decline was nearly null (dashed line). Among variant allele carriers, the association tended to deviate from linearity (solid line, $p = 0.08$), with a greater tibia Pb-associated decline in MMSE observed among men with higher tibia Pb levels. The model was adjusted for age, years of education, smoking status, pack-years smoking, nondrinker, grams/day alcohol consumption, English as first language, computer experience, and diabetes.

Source: Wang et al. (2007a).

Figure 4-8 Nonlinear association of tibia Pb level with annual rate of cognitive decline, by hemochromatosis genotype in men from the Normative Aging Study.

Evidence from Cross-sectional Studies

Associations between bone Pb levels and decrements in cognitive function in adults also are supported by evidence from several cross-sectional studies conducted in the BMS and NAS cohorts and other populations. The cross-sectional studies have contributed evidence for stronger associations of cognitive function decrements with bone Pb levels than blood Pb level and for associations with adjustment for additional potential confounding factors such as diet and medication use. While cross-sectional studies examined factors that potentially may modify risk of Pb-associated cognitive function decrements in adults, they each examined different factors and did not produce conclusive evidence regarding effect modification. These subgroup analyses also are subject to higher probability of finding an association by chance.

In addition to comparisons of blood and bone Pb levels, cross-sectional analyses of the BMS included detailed analysis of potential confounding, although smoking and alcohol use were not examined. Among 985 adults, higher tibia Pb level was associated with poorer performance in tests of language, processing speed, eye-hand coordination, executive function, verbal memory and learning, visual memory, and visuoconstruction; blood Pb level showed associations with executive function and visuoconstruction [[Table 4-10 \(Shih et al., 2006\)](#)]. Mean (SD) blood and tibia Pb levels were 3.5 (2.2) µg/dL and 18.7 (11.2) µg/g, respectively. Tibia Pb levels were associated with worse performance on tests in all domains with adjustment for age, sex, testing technician, and presence of the apolipoprotein (APO)E-ε4 allele (potential risk factor for Alzheimer's Disease). The magnitudes of associations were attenuated with additional adjustment for education, race, and household wealth; however, in these more fully-adjusted models, effect estimates for tibia Pb levels remained negative in all domains except language and processing speed. The strongest association was found for visuoconstruction, which reflects visuospatial skills and motor skills. A 1 µg/g higher tibia Pb level was associated with a 0.0044 SD lower (95% CI: -0.0091, 0.0003) visuoconstruction score. Analysis of tibia Pb as a quadratic term did not indicate a nonlinear relationship with visuoconstruction.

In contrast with longitudinal results in BMS, race-stratified analyses of persistent effects in cross-sectional analyses indicated that tibia Pb levels were associated with greater decreases in performance on tests of eye-hand coordination, executive function, and verbal memory and learning among whites than among African Americans ([Bandeem-Roche et al., 2009](#)). Among all subjects, tibia Pb-associated decrements in cognitive performance were modified by neighborhood level psychosocial stress. Specifically, higher tibia Pb levels were associated with larger decrements, particularly in language, executive function, and visuoconstruction among subjects living in neighborhoods with a greater number of psychosocial hazards (e.g., number of violent crimes, emergency calls,

off-site liquor licenses as assessed by investigators) ([Glass et al., 2009](#)) ([Table 4-10](#)). Results were adjusted for age, sex, race/ethnicity, education, testing technician, and time of day of testing. Subjects living near more psychosocial hazards had slightly higher tibia Pb levels. In support of these results, several studies have found Pb-stress interactions in impaired learning and memory of adult animals with Pb exposures beginning in gestation and lasting through post-weaning or to the time of testing ([Section 4.3.2.3](#)).

The 2006 Pb AQCD ([U.S. EPA, 2006b](#)) described cross-sectional associations of both blood and tibia Pb levels with poorer cognitive performance among 141 NAS men ([Payton et al., 1998](#)). Several recent, larger cross-sectional NAS analyses corroborated previous findings for bone Pb but generally indicated weak associations with concurrent blood Pb levels and only in groups with specific ALAD genetic variants. In contrast with the longitudinal analyses, Weisskopf et al. ([2007b](#)) found that repeat measures of bone Pb levels were inconsistently associated with cognitive function (improved and poorer performance) in cross-sectional analyses. Among 720-915 NAS men 45 years of age and older, higher concurrent blood and bone Pb levels were associated with lower MMSE scores among 149 ALAD-2 carriers than among 766 ALAD-1-1 men ([Weuve et al., 2006](#)). A 27 µg/dL higher patella Pb level (interquartile range) was associated with a 0.26 point lower mean MMSE score (95% CI: -0.64, 0.12) among ALAD-2 carriers and a 0.07 point lower score (95% CI: -0.23, 0.09) among noncarriers. Another NAS analysis (n=486-959) did not find associations of blood or bone Pb levels with cognitive function in various domains to be modified by ALAD genotype in a consistent direction ([Rajan et al., 2008](#)). The strongest interaction between higher tibia Pb level and ALAD-2 genotype was found for visuospatial skills (constructional praxis test), and between patella Pb level and ALAD-2 genotype for perceptual speed (pattern comparison test). The potential direction of effect modification by the ALAD-2 genotype is not clear as the greater affinity of the ALAD-2 enzyme subunit for Pb may increase risk of Pb-associated health effects by increasing blood Pb levels or may diminish Pb-associated health effects by sequestering Pb in the bloodstream and decreasing its bioavailability.

Cross-sectional studies examined a larger number of potential confounding factors than did longitudinal analyses. The NAS found blood and bone Pb-associated decrements in cognitive function with adjustment for dietary factors, physical activity, medication use, and comorbid conditions ([Rajan et al., 2008](#); [Weuve et al., 2006](#)) ([Table 4-10](#)). Additional potential confounding factors examined in the Nurses' Health Study of 587 healthy women in the Boston, MA area included mental health and use of aspirin, ibuprofen, antidepressants, or vitamin E. As in the BMS and NAS, tibia and patella Pb levels were more consistently associated with cognitive performance than was blood Pb level in the Nurses' Health Study ([Table 4-10](#)) ([Weuve et al., 2009](#)). Blood, patella, and tibia Pb levels were measured between ages 47 and 74 years and an average of 5 years before

cognitive testing. Contrary to expectation, higher patella and tibia Pb levels were associated with higher scores on the “f” naming test (naming words that begin with f). In separate models, the “f” naming test was omitted from a composite index of all cognitive tests performed by phone, and a 10 µg/g higher tibia Pb level was associated with 0.051-point lower (95% CI: -0.10, -0.003) composite cognitive function z-score ([Table 4-10](#)). A similar magnitude of decrease was estimated for a 3-year age increase in these women. The magnitude of association was smaller for an SD increase in patella Pb level (-0.033 [95% CI: -0.080, 0.014]), and a weak, more imprecise association was found for an SD unit increase in blood Pb level (-0.016 [95% CI: -0.071, 0.039]).

Several analyses of the large, U.S.-representative NHANES III (1991-1994) population of men and women investigated effect modification by age and genetic variants. Only blood Pb levels were available and were measured in samples collected concurrently with cognitive testing. These analyses adjusted for several of the same potential confounding factors as other studies, with the exception of smoking. Krieg and Butler ([2009](#)) found blood Pb level to be associated weakly with poorer performance on tests of learning and visuospatial skills among adults ages 40-59 years and inconsistently in adults ages 20-39 years. Krieg et al. ([2009](#)) further found inconsistent associations with word and story recall in adults ages ≥ 60 years. Because of the different types and numbers of tests administered, it is difficult to compare findings between adults less than and greater than age 60 years. In the subset of the population with genetic analysis, blood Pb-cognitive function associations were not found to be modified by ALAD genetic variants in a consistent direction ([Krieg et al., 2009](#)). Among adults ages 20-59 years and ≥ 60 years, higher concurrent blood Pb level was associated with a larger decrement in performance on some tests in ALAD-2 carriers and other tests in ALAD -1-1 subjects ([Table 4-10](#)). Krieg et al. ([2010](#)) found differences in the association between concurrent blood Pb level and cognitive scores by VDR gene polymorphisms, rs731236 and rs2239185, and by the VDR haplotype, which have unclear functional relevance. Similar to observations in adolescent NHANES participants ([Section 4.3.2.5](#)), results were not uniform across the various tests. However, for several tests, blood Pb level was associated with greater decrements in cognitive performance among adults with the CC genotypes of VDR polymorphisms.

Other cross-sectional studies with fewer subjects generally produced results consistent with those from the larger studies described above. A study of 188 rural Chinese men and women found a weak association between higher plasma Pb levels and a lower composite cognitive score based on a battery of in-person administered tests ([Gao et al., 2008](#)). Results were adjusted for age, sex, education, BMI, or APOE-ε4 in individual ANCOVA analyses. Smoking and alcohol use were not associated with cognitive performance in this group. Pb in plasma is not bound to erythrocytes, as is about 99% of blood Pb, and is

the fraction delivered directly to soft tissue ([Chuang et al., 2001](#); [Hernandez-Avila et al., 1998](#)). The results of Gao et al. ([2008](#)) may provide information on the cognitive effects of a more bioavailable fraction of Pb dose; however, because there is little investigation, firm conclusions for plasma Pb are not warranted. Among 47 men and women in Rochester, NY (ages 55-67 years), subjects in the highest tertile of calcaneal bone (heel bone with higher turnover rate than tibia) Pb level performed worse on delayed matching-to-sample and paired associated learning tasks than subjects in the lowest tertile (exact Pb levels in tertiles not reported) ([van Wijngaarden et al., 2009](#)). In analyses of tibia Pb levels, subjects in the highest tertile of tibia Pb level did not consistently perform worse on the various cognitive tests ([Table 4-10](#)).

Adults with Occupational Pb Exposures

The 2006 Pb AQCD ([U.S. EPA, 2006b](#)) concluded that in adults, blood Pb levels were associated with cognitive function more consistently among those with occupational Pb exposures. These findings were supported by results from a few recent studies of occupationally-exposed adults. Several of these associations were found with adjustment for fewer but a similar set of potential confounding factors as in nonoccupational studies; however, other occupational exposures were not considered. Occupational Mn exposure also has been linked with decrements in short-term memory and eye-hand coordination. A prospective analysis was conducted in former male Pb battery workers whose occupational exposure had ceased 0.02 to 16 years (median: 6) before follow-up testing in 2001-2004 ([Khalil et al., 2009a](#)). Subjects included 83 of 288 workers (in 2004 mean age: 54 years, median tibia Pb level: 57 µg/g) and 51 of 181 controls (mean age: 55 years, median tibia Pb level: 12 µg/g) from the 1982 Lead Occupational Study in Pennsylvania. While the follow-up participation was low, participation was not biased to poor performers on cognitive tests at baseline. In former Pb-exposed workers, a 10 µg/g higher peak tibia Pb levels was associated with a -0.352 change in total cognitive function z-score (e.g., learning/memory, executive function, general intelligence, spatial function, psychomotor speed) between 1982 and 2004. In controls, higher tibia Pb levels were associated with improved performance on several tests. Results were adjusted for age, education, income, blood pressure, years of employment, years since last worked, smoking, alcoholic drinks/week, and baseline score. Adjustment for baseline cognitive function could produce a biased association. Cross-sectional associations indicated stronger associations of concurrent tibia Pb level than concurrent blood Pb level (median: 12 µg/dL) with poorer cognitive performance in former Pb-exposed workers. Per unit increase, concurrent blood Pb levels were associated with larger decrements in cognitive performance in controls than former Pb workers. As in nonoccupationally-exposed adults, the stronger findings for tibia Pb levels in former Pb-exposed workers indicate

stronger effects of long-term cumulative Pb exposures than recent exposures on cognitive function. The associations for concurrent blood Pb levels in controls also may reflect effects of cumulative exposures.

Blood and tibia Pb levels also were associated with cognitive performance in a follow-up of 652 Pb-exposed workers (mean age: 43.4 years, mean blood Pb level: 30.9 µg/dL) in Korea, with recent results showing associations with patella Pb levels ([Dorsey et al., 2006](#)). Higher concurrent patella Pb levels were associated with poorer executive function and manual dexterity with adjustment for age, sex, education, and job duration. Associations were not found for verbal or visual memory. The associations for patella Pb level were not as strong as those previously found for either concurrent blood or baseline tibia Pb levels in these workers ([Schwartz et al., 2005](#); [2001](#)).

Other occupational studies aimed to characterize factors that either mediate or modify the association between Pb biomarkers and cognitive function. Among current Pb smelter workers, associations of a working lifetime time-weighted integrated blood Pb level (an index of cumulative exposure, mean: 42 µg/dL) ($p = 0.09$) and tibia Pb level ($p = 0.08$) with grooved pegboard test performance were mediated by Pb-associated changes in cerebral white matter ([Bleecker et al., 2007b](#)). In the same population ($n = 112$, mean age: 38 years), higher time-weighted integrated blood Pb level (mean: 34 µg/dL) was associated with decrements in executive function, learning, and memory among those with lower cognitive reserve (i.e., ≤ 11 th grade reading level by Wide Range Achievement Test-R) ([Bleecker et al., 2007a](#)). Subjects with lower and higher cognitive reserve were matched by blood Pb level (mean: 26 µg/dL), and results were adjusted for age, depression scale, and current alcohol use.

Apolipoprotein E is a transport protein for cholesterol and lipoproteins and has been found to regulate synapse formation (connections between neurons). A genetic variant, called the ApoE-ε4 allele is a haplotype between 2 exonic SNPs and has been associated with a two-fold increased risk of developing Alzheimer's disease, although the majority of such individuals still do not develop the disease. Thus, it is plausible that ApoE-ε4 carriers may be biologically susceptible to cognitive dysfunction. A study of 529 U.S. male, former tetra-ethyl Pb workers found that higher estimated peak tibia Pb levels were associated with lower scores on tests of executive function, vocabulary, and memory ([Stewart et al., 2002](#)), and for several tests, larger decrements among the 118 men with at least one ApoE-ε4 allele. Results were adjusted for age, race, education, depression score, testing technician, and visit number. Men with at least one ApoE-ε4 allele had slightly higher peak tibia Pb levels (mean: 26.2 versus 23.1 µg/g) and a larger percentage of non-white subjects but were similar in age, education, and time since employment.

Summary of Cognitive Function in Adults

In summary, consistent with evidence described in the 2006 Pb AQCD, recent studies found that higher bone Pb levels were associated decrements in cognitive function in adults without occupational Pb exposure ([Table 4-10](#)). Much of this evidence was provided by analyses of the BMS and NAS, with additional findings reported in the Nurses' Health Study and smaller populations. Nonetheless, the multiple risk factors and health outcomes examined in most of these cohorts reduces the likelihood of selection bias specifically related to Pb exposure and cognitive function. While the NAS and Nurses' Health Study included primarily white men and white women, respectively, the BMS examined a more diverse population of men and women of various races and ethnicities. There was variability in associations across the various domains of cognitive function tested within studies; however, higher bone Pb levels were associated with poorer performance in most of the tests conducted. Across populations, higher bone Pb levels were associated with decrements in executive function, visuospatial skills, learning, and memory.

Key evidence for bone Pb-associated cognitive decrements was provided by recent prospective analyses that demonstrated that higher tibia (means: 19, 20 µg/g) and patella (mean: 25 µg/g) bone Pb levels measured at baseline were associated with subsequent declines in cognitive function over 2- to 4-year periods ([Bandeem-Roche et al., 2009](#); [Weisskopf et al., 2007b](#)). These findings indicate that long-term Pb exposure may contribute to ongoing declines in cognitive function in adults. These associations were found with adjustment for potential confounding by age, education, smoking, and alcohol use in the NAS and age, sex, race, household wealth, and education in the BMS.

Supporting evidence was provided by most cross-sectional analyses, which adjusted for several of the potential confounding factors listed above plus dietary factors, physical activity, medication use, and comorbid conditions ([Rajan et al., 2008](#); [Weuve et al., 2006](#)). Cross-sectional studies generally demonstrated larger decrements in cognitive function in adults in association with tibia or patella Pb levels than with concurrent blood Pb levels. In comparisons of associations with patella and tibia Pb levels in the NAS and Nurses' Health Study, tibia Pb levels were not consistently associated with larger decreases in cognitive performance ([Weuve et al., 2009](#); [Weisskopf et al., 2007b](#)). In NHANES analyses, concurrent blood Pb levels were associated with lower cognitive function in particular age and genetic variant subgroups but not consistently across the various cognitive tests evaluated ([Krieg et al., 2010](#); [Krieg and Butler, 2009](#); [Krieg et al., 2009](#)). Analyses of NHANES did not have bone Pb measures for comparison.

Because bone Pb is a major contributor to blood Pb levels, blood Pb level also can reflect longer term exposures, including higher past exposures, especially in adults without

occupational exposures. Thus, in the NHANES results, it is difficult to characterize the relative contributions of recent and past Pb exposures to the associations observed between concurrent blood Pb level and cognitive function. In other cohorts, the discrepant findings for blood and bone Pb levels indicate that other indicators of cumulative Pb exposure (that likely included higher past exposures), may be better predictors of cognitive function in adults than is concurrent blood Pb level. Additional support for the effects of cumulative or past Pb exposure is provided by the analyses of a few child cohort as adults, which indicated that higher childhood tooth (from 1st/2nd grade) and blood (e.g., average to age 10 years) Pb levels were associated with decrements in cognitive function in adults ages 19-30 years ([Mazumdar et al., 2011](#); [Bellinger et al., 1994a](#)). An uncertainty in the evidence for bone Pb-associated decrements in cognitive function is the potential residual confounding by age. Although studies adjusted for age and/or age-squared, the high correlation between increasing age and bone Pb levels ([Section 3.3.5](#)) makes it difficult to distinguish the independent effect of Pb exposure. However, the coherence with evidence for cognitive function decrements associated with long-term Pb exposure in animals provides support for associations observed in human adults.

Cross-sectional analyses provided information on potential effect modification of bone Pb- and blood Pb-associated decrements in cognitive function in adults by race, psychosocial stress, and genetic variants. Inconsistencies were found for effect modification by race in the BMS, ALAD genotype in the NAS and NHANES, and VDR genotype in NHANES. Larger tibia Pb-associated decrements in cognitive function were found in NAS men with HFE variants and in BMS subjects living near more psychosocial hazards. Evidence does not clearly indicate whether the observed effect modification reflects chance, a change in the toxicokinetics of Pb that alters Pb dose at the biological site of action, or a direct biological interaction that increases the toxicity of Pb in the target tissue. However, such effect modification serves to strengthen inferences about associations between Pb biomarkers and cognitive function since it is unlikely that potential confounding factors vary by levels of the modifying factor, particularly genetic variants. However, because there is little available evidence and inconsistent evidence for some factors, firm conclusions regarding effect modification are not warranted.

In contrast with nonoccupationally-exposed adults, in adults with former or current occupational Pb exposures, cognitive function decrements were associated with both blood (means: 31-42 µg/dL) and bone Pb levels. Thus, among Pb-exposed workers, both current and cumulative Pb exposures may affect cognitive function. Several of these studies considered confounding by a similar set of factors as studies of nonoccupationally-exposed adults but did not consider other occupational exposures that have been linked with cognitive function decrements including Mn. In a prospective

study of former Pb workers, peak tibia Pb levels were associated more strongly with cognitive performance than were concurrent blood Pb levels ([Khalil et al., 2009a](#)). These results also indicate that in the absence of high current Pb exposures, cumulative Pb exposures may have a greater effect on cognitive function in adults.

4.3.3 Externalizing Behaviors in Children

The effects of Pb exposure on externalizing behaviors such as attention, impulsivity, hyperactivity, destructive behavior, and truancy have not been examined as extensively as effects on cognition. Similar to cognitive function, several domains of externalizing behaviors are inter-related, making it difficult to examine whether Pb exposure has an effect on a particular domain. Externalizing behaviors are more complex to study than are cognitive effects, particularly FSIQ. There are fewer objective tests of externalizing behaviors with as strong psychometric properties or as rigorous validation as IQ tests. Externalizing behaviors often are assessed using teacher and/or parent ratings which are subject to greater measurement error. However, domain-specific neuropsychological assessments are advantageous as they may provide greater insight into whether there is a particular domain more susceptible to the effects of Pb exposure.

There are three major domains of externalizing behavior disorders: attention deficit hyperactivity disorder, undersocialized aggressive conduct disorder, and socialized aggressive conduct disorder ([as reviewed in Whitcomb and Merrell, 2012](#)). Characteristics of the attention deficit hyperactivity disorder domain include but are not limited to short attention span, distractibility, impulsivity, and hyperactivity. The undersocialized aggressive conduct disorder domain can be characterized by destructive, domineering / threatening or disruptive behaviors, and irritability and explosiveness. Behaviors included within the socialized aggressive conduct disorder domain include truancy from home or school, lying and cheating, and gang membership. In the sections that follow, to the extent possible, the evaluation of evidence for externalizing behaviors is organized by the domains described above then by specific behaviors included within a domain. Although not described explicitly as a part of either domain of conduct disorders, conduct disorders can be predictors of subsequent delinquency and criminality ([Soderstrom et al., 2004](#); [Babinski et al., 1999](#); [Pajer, 1998](#)). Therefore, evidence for criminal offenses is reviewed with conduct disorders.

As with cognitive function, in the evaluation of epidemiologic evidence for externalizing behaviors, greater emphasis was placed on evidence from more rigorous neuropsychological tests, prospective studies with repeated assessments of blood Pb levels and behavior, studies of older children in whom outcomes are more reliably measured, and

studies of children whose blood Pb levels are less influenced by higher past Pb exposures. Similar to cognitive function, associations between Pb biomarker levels and externalizing behaviors potentially may be confounded by factors such as parental SES, education, and IQ, as well as quality and stability of the caregiving environment ([often evaluated as HOME score](#) [Totsika and Sylva, 2004](#)) and nutritional status. Accordingly, greater weight was given to studies with greater consideration for potential confounding in the study design or in statistical analyses. Consideration also was given to studies assessing effects relevant to blood Pb levels in current U.S. children (i.e., <5 µg/dL).

Externalizing behaviors and related clinically diagnosed disorders such as Attention Deficit Hyperactivity Disorder (ADHD) are considered to have a strong familial component ([Whitcomb and Merrell, 2012](#); [Dick et al., 2011](#); [Faraone and Doyle, 2001](#); [Kazdin, 1995](#); [Campbell and Werry, 1986](#)). In recent commentaries to studies reporting associations between blood Pb level and ADHD in children, Brondum ([2011](#), [2007](#)) asserted the need for studies to consider confounding by parental history of ADHD. Parental psychopathology potentially can affect associations between Pb and externalizing behaviors in the child through various theoretical mechanisms. One, if related only to the outcome and not to child Pb exposure, parental psychopathology can be a modifier of the child Pb exposure-externalizing behavior relationship. In this case, because the familial component of psychopathology explains a large portion of the variance in child behavior, not removing that variance with statistical adjustment could mask the smaller magnitude of risk due to other factors, including Pb. Thus, if there is an association between Pb and externalizing behaviors, it may be detected in an epidemiologic study by adjusting or stratifying by parental psychopathology. Characterization of effect modification by parental psychopathology also would depend on whether parental psychopathology is measured adequately. In the studies of Pb and externalizing behaviors, few studies have examined parental psychopathology, and none examined its role as a potential effect modifier. These studies measured parental psychopathology mostly as parental self report of ever having any among a list of many psychological, mental health, or psychiatric problems ([Cho et al., 2010](#); [Nicolescu et al., 2010](#); [Silva et al., 1988](#)) which is subject to recall error and lacks specificity for the condition being examined in the study (e.g., attention, hyperactivity, ADHD). Only Wang et al. ([2008d](#)) adjusted child ADHD associations for parent or sibling history of ADHD diagnosis, as ascertained from clinical records. Thus, in the few available studies, parental psychopathology and the familial component of externalizing behaviors likely was measured with error. However, in a potential role in effect modification, failure to account for parental psychopathology accurately or at all may attenuate or mask an association with Pb exposure if one exists.

Another way parental psychopathology potentially can influence a relationship between child Pb exposure and externalizing behaviors is by producing confounding bias. In this hypothetical case, parental psychopathology could produce an artifactual association between child Pb exposure and externalizing behaviors where there is no true association. However, this confounding bias would require that parental psychopathology not only be causally related to the outcome but also be correlated with child Pb exposure. One may speculate that parental psychopathology could affect child Pb exposure indirectly through parental caregiving quality. Parental caregiving quality is linked with externalizing behaviors in children ([Nuttall et al., 2012](#); [Campbell, 1995](#); [Rothbaum and Weisz, 1994](#); [Jacobvitz and Sroufe, 1987](#)). Theoretically, parental psychopathology may result in poorer caregiving quality or neglect which may increase a child's exposure to Pb. There is not evidence describing this link. Cho et al. ([2010](#)) found similar concurrent blood Pb levels in children with and without parental history of psychopathology, but parental report of psychopathology is subject to measurement error. Further, if parental psychopathology is a determinant of Pb exposure, then inclusion as a model covariate would adjust for Pb exposure itself and underestimate a potential association with blood Pb. Studies that examined parenting behaviors in parents with current ADHD have indicated that parents with ADHD show negative parenting control, i.e., over-reactive disciplining, lack of planning, and disorganization but have not consistently indicated that parents with ADHD have poorer emotional responsiveness, i.e., involvement with the child ([as reviewed in Johnston et al., 2012](#)). Thus, it has not been demonstrated that parents with psychopathology have poorer caregiving quality or are neglectful. Nonetheless, if caregiving quality is the mechanism by which parental psychopathology potentially can confound associations between child Pb exposure and externalizing behaviors (because of a noncausal correlation between Pb exposure and parental psychopathology), then direct adjustment for parental caregiving quality would help address potential confounding by parental psychopathology. There still is the possibility of residual confounding by parental caregiving quality if not measured accurately, but this applies to any potential confounding factor examined for any outcome in relation to any exposure. Research has not established a direct relationship between parental psychopathology and child Pb exposure or one between parental psychopathology and poorer parental caregiving quality. Thus, based on the currently available evidence, in this ISA, parental psychopathology itself is not considered to be a potential confounder of associations between child Pb and externalizing behaviors. Instead, the ISA evaluates potential confounding by parental caregiving quality in addition to other factors well documented in the literature to be correlated with both Pb exposure and externalizing behaviors as described earlier in this section.

4.3.3.1 Attention, Impulsivity, and Hyperactivity in Children

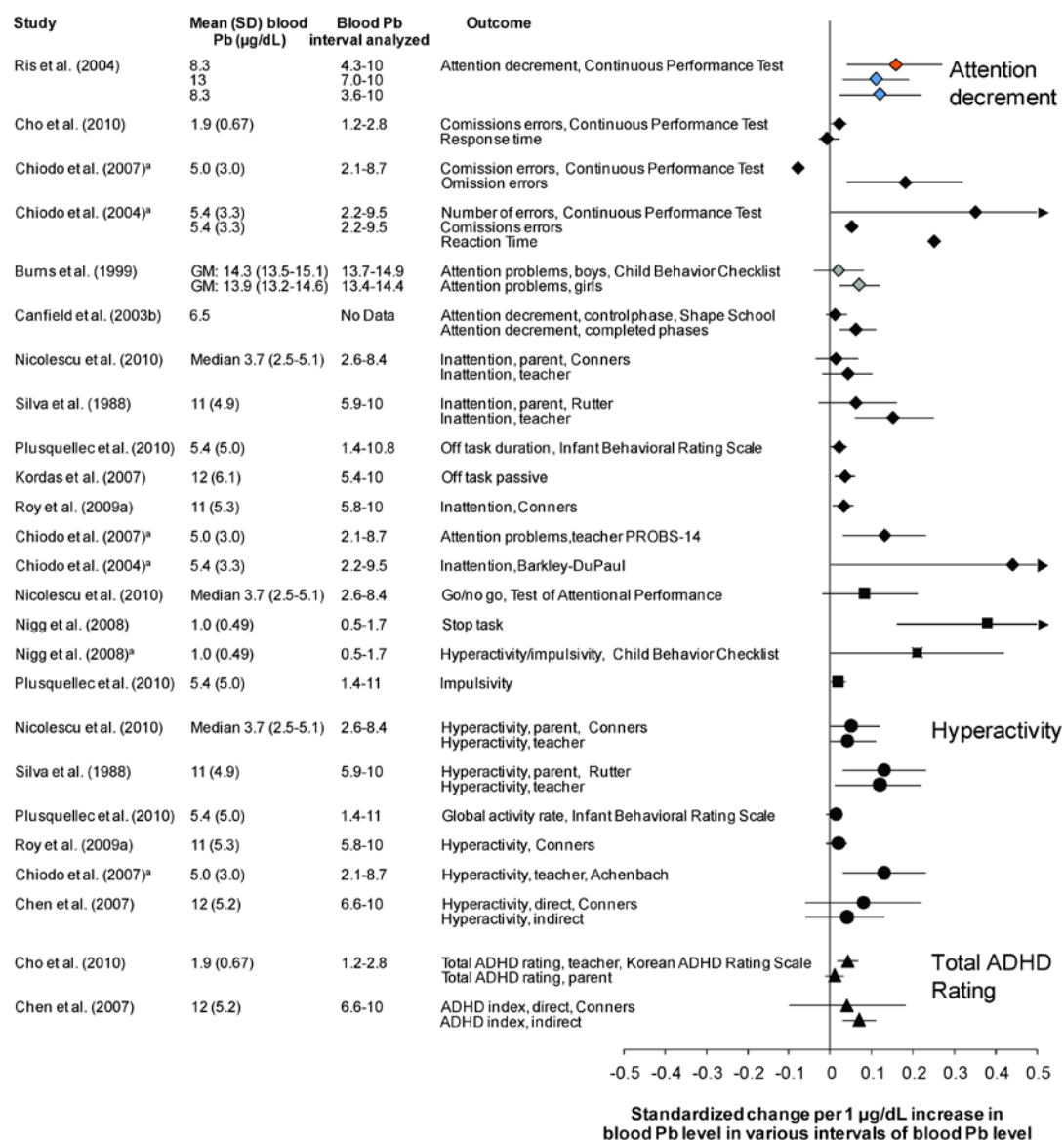
Within the attention deficit hyperactivity disorder domain of externalizing behaviors, studies of Pb exposure have focused primarily on attention, impulsivity, and hyperactivity. Some epidemiologic studies have examined a composite index of multiple behaviors, and a few studies have examined physician-diagnosed ADHD. The evidence for each of these outcomes is described below individually to the extent possible. Tests in animals can measure aspects of both attention and impulsivity; however, in several tests, a particular behavior that is observed can be identified as predominately reflecting a decrease in attention or increase in impulsivity. Therefore, the animal evidence is discussed separately according to the dominant process inferred to characterize a particular behavior measured by a neuropsychological test.

Epidemiologic Studies of Attention in Children

Among the various externalizing behaviors examined with Pb, most of the evidence for a relationship with Pb exposure is for measures of attention. Attention is the ability to maintain a consistent focus on an activity or relevant stimuli and can be assessed by examining sustained attention, concentration or distractibility. Several epidemiologic studies reviewed in the 2006 Pb AQCD ([U.S. EPA, 2006b](#)) reported associations between higher blood or tooth Pb levels with attention decrements in children from 1st grade to age 17 years, including prospective studies described in previous sections for cognitive function ([Ris et al., 2004](#); [Fergusson et al., 1993](#); [Leviton et al., 1993](#)). As described in this section, most recent studies also found associations of higher blood Pb levels with attention decrements in children ages 7-17 years. Many previous studies of attention included children with higher blood Pb levels than most current U.S. children but had strengths including prospective design and greater consideration for potential confounding. Some recent studies found blood Pb-associated attention decrements in populations of children with mean concurrent blood Pb levels 2 to 5 µg/dL ([Cho et al., 2010](#); [Nicolescu et al., 2010](#); [Plusquellec et al., 2010](#); [Chiodo et al., 2007](#)) but in contrast with prospective studies had limitations including cross-sectional design, the potential influence of higher past Pb exposures, and consideration of fewer potential confounding factors. In the collective body of literature, most evidence was for attention rated by teachers, parents, or blinded examiners; however, associations were consistently found for more objective measures such as performance on the continuous performance test (CPT) ([Figure 4-9](#) and [Table 4-11](#)). Thus, evidence does not indicate undue influence by biased reporting of attention decrements by parents of children with higher Pb exposures. In support of the epidemiologic findings for attention decrements in children, some

animal studies have found Pb exposure to induce less accurate signal detection in tests with distracting stimuli, which can reflect impaired attention (discussed below).

Most prospective studies that examined attention found associations with blood or tooth Pb level and had several strengths. With prenatal blood Pb, blood Pb measured 9-11 years before attention, lifetime average blood Pb, or tooth Pb, prospective studies characterized the temporal sequence between Pb exposure and attention decrements better than cross-sectional studies and made reverse causation a less likely explanation for the observed associations. These studies recruited cohorts from schools or prenatal clinics and had moderate to high follow-up participation that was not conditional on blood or tooth Pb levels, which reduces the likelihood of selection bias. Not all results were statistically significant but overall they showed a pattern of lower attention with higher blood or tooth Pb level ([Figure 4-9](#) and [Table 4-11](#)). Most of these prospective studies were reviewed in the 2006 Pb AQCD and examined populations with mean blood Pb levels 7-14 µg/dL.



^aStandard errors were estimated from p-values or sufficient data were not available to calculate 95% CIs.

Note: Regression coefficients were reported as T- or factor scores or were scaled to their standard deviation to facilitate comparisons among tests with different scales. Small effect estimates do not necessarily indicate lack of effect or weak effect. Results are categorized by outcome category. Where available, results from objective tests are presented first. Within categories, results generally are grouped according to strength of study design, representativeness of the study population characteristics and blood Pb levels examined, and extent of potential confounding considered. There is not necessarily a continuum of decreasing strength across studies. Effect estimates are standardized to a 1 µg/dL increase in blood Pb level in the interval from the 10th to the 90th percentile, or to 10 µg/dL, whichever is lower. The percentiles are estimated using various methods and are approximate values. Effect estimates are assumed to be linear within the interval of blood Pb level examined. Orange, blue, black, and gray symbols represent associations with prenatal maternal, childhood several years before outcome, concurrent, and lifetime average blood Pb levels, respectively.

Figure 4-9 Associations of blood Pb levels with attention, impulsivity, and hyperactivity in children.

Table 4-11 Additional characteristics and quantitative results for studies presented in Figure 4-9.

Study	Study Population and Methodological Details Organized by outcome then strength of study methodology, generalizability ^a	Blood/Tooth Pb Metrics Analyzed (µg/dL or µg/g)	Outcomes Analyzed	Effect Estimate (95% CI) ^b
Bellinger et al. (1994a)	79 young adults followed from 1st-2nd grade (1975-1978) to ages 19-20 yr, Chelsea/Somerville, MA Prospective. Low follow-up participation. Participation from higher SES and initial IQ and nonwhite but no effect on association with tooth Pb level. Log linear regression model adjusted for parental IQ, sex, SES, current drug, alcohol and illicit drug use, maternal education and age, birth order. Also considered potential confounding by other unspecified factors.	Deciduous tooth (1st/2nd grade) in ppm Q1: 2.9-5.9 Q2: 6.0-8.7 Q3: 8.8-19.8 Q4: 19.9-51.8	Mean (SE) Correct Responses per quartile Mean (SE) Reaction Time Errors per quartile Continuous Performance Test (CPT), ages 19-20 yr	Q1: 98.0 (1.0) ^c Q2: 97.6 (1.1) Q3: 96.9 (1.1) Q4: 94.6 (1.1) Q1: 361.2 (16.5) Q2: 374.1 (17.3) Q3: 370.7 (17.9) Q4: 385.0 (17.1)
Ris et al. (2004)	195 children followed prenatally (1979-1985) to age 15-17 yr, Cincinnati, OH Prospective. Recruitment at prenatal clinic. High follow-up participation, no selective attrition. Mostly African-American. Linear regression model adjusted for SES, maternal IQ, HOME score, adolescent marijuana use, child sex, birth weight. Also considered potential confounding by birth outcomes, maternal age, prenatal smoking, alcohol, marijuana, and narcotics use, gravidity, # previous abortions, stillbirths, parity, caregiver education, public assistance, child age, health, and Fe status.	Mean (SD) Prenatal maternal: 8.3 (3.8) Age 3-60 mo avg: 13 (6.1) Age 78 mo: 8.3 (4.8) See Wright et al. (2008) Intervals analyzed: 4.3, 7.0, 3.6 (10th percentiles) -10	Decrement in attention factor of CPT outcomes, ages 15-17 yr Prenatal (maternal) Age 3-60 mo avg Age 78 mo	 0.16 (0.04, 0.27) 0.11 (0.03, 0.19) 0.12 (0.02, 0.22)
Cho et al. (2010)	639 children ages 8-11 yr, born 1997-2000, Seoul, Seongnam, Ulsan, Incheon, Yeoncheon, Korea Cross-sectional. School-based recruitment, moderate participation rate. Log linear regression model adjusted for age, sex, paternal education, maternal IQ, child IQ, birth weight, urinary cotinine, residential area. Did not consider potential confounding by parental caregiving quality.	Concurrent blood Mean (SD): 1.9 (0.67) Interval analyzed: 1.2-2.8 = 10th-90th percentiles	Commission errors, CPT Response time, CPT Total ADHD rating, teacher Total ADHD rating, parent Korean ADHD Rating Scale IV Ages 8-11 yr	0.02 (0, 0.04) ^d -0.01 (-0.03, 0.02) ^d 0.04 (0.02, 0.07) ^d 0.01 (-0.01, 0.03) ^d

Table 4-11 (Continued): Additional characteristics and quantitative results for studies presented in Figure 4-9.

Study	Study Population and Methodological Details Organized by outcome then strength of study methodology, generalizability ^a	Blood/Tooth Pb Metrics Analyzed (µg/dL or µg/g)	Outcomes Analyzed	Effect Estimate (95% CI) ^b
Nicolescu et al. (2010)	83 children ages 8-12 yr (born 1995-1999), Bucharest and Pantelimon, Romania Cross-sectional. Pantelimon near former metal processing plant. Low correlations blood Pb with blood Al, Hg. No information on participation rate. Log linear regression model adjusted for city, sex, age, computer experience, handedness, eye problems, # siblings, parental education, prenatal smoking and alcohol use, parent ever having psychological/psychiatric problem (for go/no go).	Concurrent blood Median (25th-75th): 3.7 (2.5-5.1) Interval analyzed: 2.6-8.4 = 10th-90th percentiles	Go/no go, KITAP Inattention parent rating Inattention teacher rating Hyperactivity, parent rating Hyperactivity, teacher rating German Conners Rating Ages 8-12 yr	8.2% (-0.02, 21) ^e 1.2% (-3.5, 6.7) 4.1% (-0.02, 10) 5.0% (0, 12) 4.1% (-0.02, 11)
Nigg et al. (2008)	150 children ages 8-17 yr, Birth yr and location not reported Case-control study of ADHD. Recruitment by mailings, outreach to clinics, community advertisements. Could have biased participation by Pb exposure leading to biased behavioral ratings. Regression-based path analysis adjusted for sex and income. Did not consider potential confounding by parental education or caregiving quality.	Concurrent blood Mean (SD): 1.03 (0.49) Detection limit=0.3 Interval analyzed: 0.5-1.7 = 10th-90th percentiles	Stop task Hyperactivity/impulsivity Teacher, parent rating, Child Behavioral Checklist, ADHD Rating Scale, Conners Rating Scale Ages 8-17 yr	0.38 (0.16, 0.60) ^d 0.21 (0, 0.42) ^d
Needleman et al. (1979)	158 children in 1st/2nd grade (1975-1978), Chelsea, Somerville, MA Cross-sectional. Recruitment from schools. Only 15% selected based on low and high tooth Pb levels. Low participation rate but no selective participation based on tooth Pb or teacher ratings. Analysis of covariance adjusted for paternal SES, parental IQ, and maternal age, # pregnancies, and education. Did not consider potential confounding by parental caregiving quality.	Tooth (1st/2nd grade) High >20 ppm (n = 58) Low <10 ppm (n = 100)	Reaction time, 12 sec delay % negative response, teacher rating Impulsive Hyperactive	Mean (SD) seconds ^c Low tooth Pb: 0.41 (0.09) High tooth Pb: 0.47 (0.12) High vs. low tooth Pb 25 vs. 9%, p = 0.01 16 vs. 6%, p = 0.08

Table 4-11 (Continued): Additional characteristics and quantitative results for studies presented in Figure 4-9.

Study	Study Population and Methodological Details Organized by outcome then strength of study methodology, generalizability ^a	Blood/Tooth Pb Metrics Analyzed (µg/dL or µg/g)	Outcomes Analyzed	Effect Estimate (95% CI) ^b
Chiodo et al. (2007)	451-466 children, age 7 yr (born 1989-1991), Detroit, MI area. Cross-sectional. Recruitment at prenatal clinic. 100% African-American. High prevalence prenatal drug exposure. High participation rate. Linear regression model adjusted for child sex, prenatal marijuana use (commission errors), caregiver education, HOME, maternal IQ, cocaine use, prenatal alcohol use (omission errors), child age and sex (hyperactivity), child age and sex, SES, HOME, caretaker education, maternal age, prenatal alcohol use, current marijuana use (attention problems). Also considered potential confounding by # children in home, caretaker marital status, concurrent alcohol/week and cigarettes/day, caregiver concurrent psychological symptoms, and maternal custody and prenatal cigarettes/day	Concurrent blood Mean (SD): 5.0 (3.0) Interval analyzed: 2.1-8.7 = 10th-90th percentiles	Commission Errors (%), CPT	-0.08, p >0.05 ^f
			Omission Errors (%), CPT	0.18 (0.04, 0.32) ^d
			Attention problems, teacher rating, PROBS-14	0.13 (0.03, 0.23) ^d
			Hyperactivity, Achenbach Teacher Report Form Age 7 yr	0.13 (0.03, 0.23) ^d
Chiodo et al. (2004)	164-236 children, age 7.5 yr, Detroit, MI area Cross-sectional. Recruitment at prenatal clinic. 100% African-American. High prevalence prenatal alcohol and drug exposure. Moderate participation rate. Log linear regression model adjusted for SES, # children <age 18 yr caregiver vocabulary (# errors CPT); SES, caregiver vocabulary, child age (commission errors, CPT); caregiver education, child sex, and prenatal cocaine exposure (reaction time); HOME, child sex, prenatal alcohol exposure (Inattention rating). Also considered potential confounding by family functioning, maternal prenatal marijuana or smoking exposure, parity, crowding, child life stress, caregiver age, life stress, and psychopathology, conflict tactics.	Concurrent blood Mean (SD): 5.4 (3.3) Interval analyzed: 2.2-9.5 = 10th-90th percentiles	Number of errors, visual CPT	0.35 (0, 0.69) ^d
			Commission errors, visual CPT	0.05, p >0.05 ^f
			Reaction time, visual CPT	0.25, p >0.05 ^f
			Inattention Factor, Barkley-DuPaul Scale Age 7.5 yr	0.44 (0, 0.89) ^d

Table 4-11 (Continued): Additional characteristics and quantitative results for studies presented in Figure 4-9.

Study	Study Population and Methodological Details Organized by outcome then strength of study methodology, generalizability ^a	Blood/Tooth Pb Metrics Analyzed (µg/dL or µg/g)	Outcomes Analyzed	Effect Estimate (95% CI) ^b
Fergusson et al. (1993)	878 children followed from birth to age 13 yr, Christchurch, New Zealand Prospective. Moderate follow-up participation, attrition did not affect results. Log linear regression model adjusted for sex, maternal education, family size, HOME score, # schools attended. Also considered potential confounding by ethnicity, SES, maternal age, breastfeeding duration, paternal education, parental smoking, child birth outcomes, residence on busy roads, weatherboard housing.	Tooth (age 6-8 yr) Mean (SD): 6.2 (3.7) µg/g	Inattention/restlessness and Conners', parent and teacher ratings Age 13 yr	0.06 (0, 0.12) ^{c,d}
Chandramouli et al. (2009)	488 children followed from age 4 mo (born 1992) to ages 7-8 yr, Avon, U.K. Prospective. All births in area eligible. Similar characteristics as U.K. census, high participation at baseline and follow-up. Participants had better educated mothers, who smoked less, better home environment. Log linear regression model adjusted for maternal education and smoking, home ownership, home facilities score, family adversity index, paternal SES, parenting attitudes at 6 mo, child sex. Also considered potential confounding by child IQ.	Age 30 mo blood Not reported		OR for increased score
		Group 1: 0-<2	Selective attention decrements	Reference
		Group 2: 2-<5	Test of Everyday Attention for Children Ages 8 yr	1.03 (0.66, 1.61) ^c
		Group 3: 5-<10		0.99 (0.62, 1.57) ^c
		Group 4: >10		1.14 (0.54, 2.40) ^c
		Group 1: 0-<2	Hyperactivity, teacher	Reference
		Group 2: 2-<5	Strengths and Difficulties	0.84 (0.47, 1.52) ^c
		Group 3: 5-<10	Questionnaire, Ages 7 yr	1.25 (0.67, 2.33) ^c
		Group 4: >10		2.82 (1.08, 7.35) ^c
Leviton et al. (1993)	1,923 children followed from birth (1979-1980) to age 8 yr, Boston, MA area Prospective. Recruited from birth hospital. Moderate participation at baseline and follow-up. Regression model adjusted for single parent family, gestational age, # children in home, maternal education, ethnicity, daycare in 1st 3 years. Also considered potential confounding by other unspecified factors	Prenatal (cord) blood Mean: 6.8 Tooth Pb (Age 6 yr) Mean: 3.3 µg/g	Daydreaming problems, Boston Teacher Questionnaire, age 8 yr Prenatal (cord) Tooth Pb	RR (yes/no) per natural log increase Girls: 1.3 (0.8, 2.2) ^c Boys: 1.0 (0.6, 1.5) ^c Girls: 1.5 (0.9, 2.6) ^c Boys: 1.1 (0.7, 1.7) ^c

Table 4-11 (Continued): Additional characteristics and quantitative results for studies presented in Figure 4-9.

Study	Study Population and Methodological Details Organized by outcome then strength of study methodology, generalizability ^a	Blood/Tooth Pb Metrics Analyzed (µg/dL or µg/g)	Outcomes Analyzed	Effect Estimate (95% CI) ^b
Burns et al. (1999)	322 children followed from birth (1979-1982) to ages 11-13 yr, Port Pirie, Australia. Prospective. Moderate follow-up participation. Participants had higher birth weight, older mothers, less educated fathers. Log linear regression model adjusted for maternal age, prenatal smoking status, IQ, concurrent psychopathology, and education, birth weight, type of feeding, length of breastfeeding, paternal education and occupation, birth order, family functioning, parental smoking, marital status, HOME score, child IQ.	Lifetime avg (to age 11-13 yr) blood GM (5th-95th) Boys: 14.3 (13.5-15.1) 10th-90th: 13.7-14.9 Girls: 13.9 (13.2-14.6) 10th-90th: 13.4-14.5	Attention problems, boys Attention problems, girls Maternal rating by Child Behavior Checklist Ages 11-13 yr	0.02 (-0.04, 0.08) 0.07 (0.02, 0.12)
Wasserman et al. (2001)	191 children followed prenatally (1984-1985) to age 5 yr, Pristina, Yugoslavia. Prospective. High follow-up participation. Participants had lower maternal education, were Albanian, had higher average blood Pb at age 4 yr. Generalized estimating equations with log blood Pb adjusted for child sex, birth weight, and age, ethnicity, HOME score, maternal education and smoking. Did not consider potential confounding by maternal IQ.	Lifetime avg (to age 5 yr) blood Mean (SD): 7.2 Only log blood Pb reported	Attention problems Maternal rating, Child Behavior Checklist Repeated measures ages 4-5 yr.	Change in raw score per log increase in blood Pb: 0.06 (-0.21, 0.33) ^c
Chen et al. (2007)	721 children in TLC trial followed from ages 1-3 to 5 yr, Baltimore, MD; Cincinnati, OH; Newark, NJ; Philadelphia, PA Prospective. Mostly African-American. Multi-city, high participation rate. Eligible for chelation trial because of high age 1-3 yr blood Pb levels (20-44 µg/dL). Linear regression-based path analysis adjusted for city, race, sex, language, single parent, parental education and employment, age at blood Pb measurement, caregiver IQ. Considered potential confounding by chelation treatment but not parental caregiving quality. Direct = independent of IQ. Indirect = mediated through IQ.	Age 2 year blood Mean (SD): 26 (5.1) Interval analyzed: 20-33 =10th-90th percentiles Concurrent blood Mean (SD): 12 (5.2) Interval analyzed: 6.6 (10th percentile)-10	Hyperactivity Index ADHD index Hyperactivity Index ADHD index Parent ratings, Conners Scale-Revised, Age 5 yr	Direct: 0.07 (-0.07, 0.20) Indirect: 0.03 (0, 0.05) Direct: 0.04 (-0.09, 0.18) Indirect: 0.05 (0.03, 0.13) Direct: 0.08 (-0.06, 0.21) Indirect: 0.04 (-0.06, 0.13) Direct: 0.04 (-0.10, 0.18) Indirect: 0.07 (0.03, 0.11)

Table 4-11 (Continued): Additional characteristics and quantitative results for studies presented in Figure 4-9.

Study	Study Population and Methodological Details Organized by outcome then strength of study methodology, generalizability ^a	Blood/Tooth Pb Metrics Analyzed (µg/dL or µg/g)	Outcomes Analyzed	Effect Estimate (95% CI) ^b
Canfield et al. (2003b)	96-127 children born 1994-1995 followed ages 6 mo-4-4.5 yr, Rochester, NY Cross-sectional. Recruitment from study of dust control. Mostly nonwhite. Moderate follow-up participation, no comparison of nonparticipants. Linear mixed effects model adjusted for child age, gestational age, maternal IQ and education, HOME, race, color/shape knowledge, child IQ (both outcomes). Plus: race (control phase), child sex (inhibit phase). Also considered potential confounding by birth weight, household income, prenatal smoking exposure.	Age 4 yr blood Mean: 6.5 Range: 1.7-21 10th-90th: data not available	Attention decrement, control phase Attention decrement, inhibit phase Examiner rating during Shape School Task Repeated measures at ages 4 and 4.5 yr	0.01 (-0.01, 0.04) 0.008 (-0.02, 0.04)
Silva et al. (1988)	535 children age 11 yr (born 1972-1973), Dunedin, New Zealand Cross-sectional. Moderate participation rate. Participants were of higher SES and non-Maori. Log linear regression adjusted for SES, maternal verbal skills, age at 1 st birth, and mental health symptoms, change in residence and school, solo parenting, child/parent separation, family relations, marriage guidance sought, child sex, birth order, and IQ. Did not consider potential confounding by parental caregiving quality.	Concurrent blood Mean (SD): 11 (4.9) Interval analyzed: 5.9 (10th percentile)-10	Inattention, parent Inattention, teacher Hyperactivity, parent Hyperactivity, teacher Rutter Behavior Questionnaire Age 11 yr	0.06 (-0.03, 0.16) 0.15 (0.06, 0.25) 0.13 (0.03, 0.23) 0.12 (0.01, 0.22)
Plusquellec et al. (2010)	84-98 children, ages 5-6 years (born 1994-1997), Inuit communities, Quebec, Canada Cross-sectional. Study of multiple exposures. Low but no selective participation by blood Pb, PCBs, or Hg. Log linear regression model adjusted for birth weight, sex, parity, caregiver education (impulsivity); birth weight, SES, child blood Hg (off task duration), maternal prenatal binge drinking, # children in home, cord and child serum fatty acids (global activity rate). Also examined potential confounding by, # residents per room, caretaker psychological distress, nonverbal reasoning, and linguistic acculturation, HOME, maternal prenatal illicit drug use and cigarettes/day, serum Se. $p > 0.20$ for associations with blood Hg or PCBs.	Concurrent blood Mean (SD): 5.4 (5.0) Interval analyzed: 1.4-11 = 10th-90th percentiles	Off task duration Impulsivity Global activity rate Examiner ratings, modified Infant Behavior Rating Scale Ages 5-6 yr	0.02 (0, 0.04) ^d 0.02 (0, 0.04) ^d 0.014 (-0.01, 0.03) ^d

Table 4-11 (Continued): Additional characteristics and quantitative results for studies presented in Figure 4-9.

Study	Study Population and Methodological Details Organized by outcome then strength of study methodology, generalizability ^a	Blood/Tooth Pb Metrics Analyzed (µg/dL or µg/g)	Outcomes Analyzed	Effect Estimate (95% CI) ^b
Kordas et al. (2007)	157 children ages 6-8 yr (born 1993-1995), Torreon, Mexico Cross-sectional. 26% selected from larger study for classroom observation. Residence near metal foundry. Linear regression model adjusted for age, sex, SES, home ownership, crowding in home, maternal education, child living with 1 or both parents, forgetting homework. Also considered potential confounding by micronutrients but not parental caregiving quality.	Concurrent blood Mean (SD): 12 (6.1) Interval analyzed: 5.4 (10th percentile)-10	Off task passive behavior Examiner rating, instrument developed by investigator Ages 6-8 yr	0.034 (0.01, 0.06)
Roy et al. (2009)	756 children ages 3-7 yr (born 1998-2003), Chennai, India Cross-sectional. Recruitment at schools. No information provided on participation. Log linear regression model adjusted for age, sex, average monthly income, hemoglobin, parental education, # other children, cluster of school and classroom. Did not consider potential confounding by parental caregiving quality.	Concurrent blood Mean (SD): 11 (5.3) Interval analyzed: 5.8 (10th percentile)-10	Inattentive z-score Hyperactive z-score ADHD index z-score Teacher ratings, Conners' ADHD/DSM-IV Scales Ages 3-7 yr	0.03 (0.01, 0.06) 0.02 (-0.01, 0.04) 0.02 (0, 0.05)
Rabinowitz et al. (1992)	493 children, grades 1-3, Taiwan Cross-sectional. Some reside near smelter. Recruitment from schools. High participation rate. Logistic regression model adjusted for sex, # adults at home. Also considered potential confounding by grade, child longest hospital stay parental education, SES, birth outcomes, handedness, language at home, prenatal maternal medicine, alcohol, and smoking but not parental caregiving quality.	Tooth (grades 1-3) Mean (SD): 4.6 (3.5)	Hyperactivity Syndrome Boston Teacher Questionnaire Grades 1-3	OR vs. <2.3 µg/g as reference 2.3-7 µg/g: 1.9 (0.53, 6.5) ^c >7 µg/g: 2.8 (0.68, 12) ^c

Table 4-11 (Continued): Additional characteristics and quantitative results for studies presented in Figure 4-9.

Study	Study Population and Methodological Details Organized by outcome then strength of study methodology, generalizability ^a	Blood/Tooth Pb Metrics Analyzed (µg/dL or µg/g)	Outcomes Analyzed	Effect Estimate (95% CI) ^b
Froehlich et al. (2009)	2,588 children, ages 8-15 yr (born 1986-1996), U.S. NHANES 2001-2004 Cross-sectional. U.S. representative results, study of multiple risk factors and outcomes. Logistic regression adjusted for child serum cotinine, sex, age, preschool attendance, birth weight, income/poverty ratio, maternal age and prenatal smoking, race/ethnicity. Did not consider potential confounding by parental education or caregiving quality.	Concurrent blood Tertile 1: 0.2-0.8 Tertile 2: 0.9-1.3 Tertile 3: >1.3 1% below detection limit of 0.3	ADHD DSM-IV criteria met (yes/no) Parental rating, ADHD module of Diagnostic Interview Schedule for Children Age 8-15 yr	OR vs. 0.2-0.8 µg/dL as reference 0.9-1.3 µg/dL: 1.7 (0.97, 2.9) ^g >1.3 µg/dL: 2.3 (1.5, 3.8) ^g

^aResults are organized by outcome examined, then grouped by strength of study design, representativeness of the study population characteristics and blood Pb levels examined, and extent of potential confounding considered. There is not necessarily a continuum of decreasing strength across studies.

^bEffect estimates are standardized to a 1 µg/dL increase in blood Pb level in the interval from the 10th percentile of blood Pb level to 10 µg/dL or the 90th percentile, whichever is lower and scaled to the standard deviation of the test score to facilitate comparisons among tests that are scored on different scales. For studies with 10th percentiles of blood Pb level > 10 µg/dL, effect estimates are standardized to a 1 µg/dL increase in blood Pb level in the interval from the 10th to 90th percentile of blood Pb level.

^cResults not presented in [Figure 4-9](#) because tooth Pb analyzed or data reported only for log blood Pb.

^dStandard error was estimated from the reported p-value.

^eResults represent the change in false alarm rate.

^fSufficient data were not available to calculate 95% CIs.

^gResults not presented in [Figure 4-9](#) because OR or RR reported in papers.

Prospective studies in Cincinnati and in Chelsea/Somerville, MA found Pb-associated attention decrements in adolescents and young adults as indicated by increases in commission and omission errors or reaction time on the objective CPT ([Ris et al., 2004](#); [Bellinger et al., 1994a](#)). In the CPT, subjects are assessed for their ability to maintain focus during a repetitive task and respond to targets or inhibit responses. Stronger, more consistent results were found in the larger Cincinnati cohort with the analysis of blood Pb levels as a continuous variable. In 195 mostly African-American, lower SES adolescents ages 15-17 years, Ris et al. ([2004](#)) found attention decrements (composite of CPT outcomes) in association with higher prenatal maternal, age 3-60 month average, and age 78 month blood Pb levels, particularly among males. The mean (SD) blood Pb levels as reported in Wright et al. ([2008](#)) were 8.3 (3.8) µg/dL for maternal prenatal, 13.4 (6.1) for age 3-60 month average, and 8.3 (4.8) for age 78 month. Among 79 primarily white, higher SES young adults ages 19-20 years, Bellinger et al. ([1994a](#)) found that compared with the group with 1st/2nd grade tooth Pb levels 2.9-5.9 ppm, the group with tooth Pb levels >19.9 ppm had fewer correct responses on the CPT and had longer reaction times for correct responses but did not commit more commission errors (responding to a nontarget) ($p = 0.25$ for overall sustain factor). Although blood Pb levels at older ages were not examined and results do not exclude an effect of more recent Pb exposures, the combined evidence from these prospective CPT studies of points to an effect on attention of Pb exposures from earlier in development.

In addition to objective tests of attention, most prospective studies found Pb-associated attention decrements in school-aged children as rated by teachers, parents, or independent examiners ([Figure 4-9](#) and [Table 4-11](#)). In children ages 7-13 years in Australia, New Zealand, and Boston, MA, lower ratings of attention were associated with higher lifetime (to age 11-13 years) average ([Burns et al., 1999](#)), prenatal cord ([Leviton et al., 1993](#)), and tooth (collected at ages 6-8 years) Pb levels ([Fergusson et al., 1993](#); [Leviton et al., 1993](#)). The mean blood Pb levels in these populations were 13.9 µg/dL for lifetime average ([Burns et al., 1999](#)) and 6.8 µg/dL for prenatal cord ([Leviton et al., 1993](#)). In separate cohorts, associations of lifetime average blood Pb level ([Burns et al., 1999](#)) and tooth Pb level ([Leviton et al., 1993](#)) with attention ratings were stronger among girls than boys. Other prospective studies found lack of association with early childhood or lifetime average blood Pb levels. Age 30 month blood Pb levels was not associated with teacher rating of selective inattention in U.K. children ages 7-8 years ([Chandramouli et al., 2009](#)). Lifetime average blood Pb level was not associated with maternal rating of attention problems in the Yugoslavia cohort at age 4-5 years ([Wasserman et al., 2001](#)). However, ratings may be less reliable in younger children, in whom patterns of behavior are less well established.

Another strength of prospective studies, both those finding and not finding associations, was their examination of many potential confounding factors, either by inclusion in statistical models with Pb or by examination in preliminary analyses. Higher blood or tooth Pb levels were associated with attention decrements with adjustment for parental education, IQ, and caregiving quality as well as SES in most studies ([Ris et al., 2004](#); [Burns et al., 1999](#); [Fergusson et al., 1993](#)) and in fewer studies, birth outcomes and exposures to smoking, alcohol, or drugs. These results increase confidence that the associations observed with Pb biomarkers reflect a relationship with Pb exposure.

Most cross-sectional studies found associations between higher concurrent blood, tooth, or hair Pb level and attention decrements among children ages 3-16 years. With analysis limited to concurrently measured Pb biomarkers, the directionality of effects is difficult to characterize. While these studies considered potential confounding by SES, child age, sex, family structure, or parental education, most did not examine potential confounding by parental caregiving quality. Cross-sectional studies comprised a mix of previously reviewed and recently published studies and a range of concurrent blood Pb levels, means 1-13.2 µg/dL ([Table 4-11](#)). Among studies of children with mean concurrent blood Pb levels 11.1-13.2 µg/dL, most ([Roy et al., 2009](#); [Kordas et al., 2007](#); [Silva et al., 1988](#)) but not all ([Liu et al., 2011b](#)) found associations with lower attention ratings. Associations with blood or hair Pb levels were found in most of the study populations that lived near metal sources in Mexico or China ([Liu et al., 2011b](#); [Bao et al., 2009](#); [Kordas et al., 2007](#)). Needleman et al. ([1979](#)) found longer reaction time at varying intervals of delay and higher ratings of distractibility in children in grades 1-2 in Chelsea and Somerville, MA with tooth Pb levels > 24 ppm than those with tooth Pb <6 ppm ([Table 4-11](#)). Some studies included young children (e.g., ages 3, 4 years), in whom behavioral ratings may be less reliable ([Liu et al., 2011b](#); [Roy et al., 2009](#)).

Evidence of Pb-associated attention decrements in populations with mean concurrent blood Pb levels 1-6.5 µg/dL (ages 4-17 years) was more variable. A large group of these studies considered several potential confounding factors including SES, parental education, parental IQ, and parental caregiving quality ([Plusquellec et al., 2010](#); [Chiodo et al., 2007](#); [2004](#); [Canfield et al., 2003b](#)). These studies also had population-based recruitment and moderate to high follow-up, without indication of biased participation by children with higher blood Pb levels and poorer attention. However, in these higher-quality studies of children with lower blood Pb levels, there was a difference in consistency of evidence between studies of younger children ages 4-5 years and older children ages 7 years. Chiodo et al. ([2007](#); [2004](#)) examined children ages 7 years in Detroit, MI and found concurrent blood Pb-associated poorer performance on several tests of attention on the CPT and lower teacher or study examiner ratings of attention. In these populations with high prevalence of prenatal alcohol or drug exposure, results did

not indicate confounding by these other exposures. However, the presence of these other exposures may limit the generalizability of results.

Among the lower concurrent blood Pb level studies that included younger children, ages 4-5 years, evidence was mixed. An association between higher concurrent blood Pb level and attention decrements was found among Inuit children ages 5-6 years in Quebec, Canada ([Plusquellec et al., 2010](#)) but not the Rochester cohort ([Canfield et al., 2003b](#)). These results were based on maternal or study examiner ratings. Ratings may be less reliable in younger children, in whom patterns of behavior are less well established. Canfield et al. ([2003b](#)) initially found associations between higher concurrent blood Pb level and lower ratings of attention, but they were attenuated with adjustment for child color and shape knowledge and age 3-year FSIQ, which suggested that poorer knowledge of the task parameters may increase distraction. However, Cho et al. ([2010](#)) found an association between concurrent blood Pb level and lower attention ratings with adjustment for child IQ, indicating the relationship between attention and cognitive function may vary across populations. In the study of Inuit children, concurrent blood Pb level but not cord blood Pb level was associated with duration of off-task behavior as rated by study examiners ([Plusquellec et al., 2010](#)). In this population with potential co-exposures to polychlorinated biphenyls (PCBs) and mercury (Hg) from high consumption of fish, blood levels of PCBs and Hg were reported not to be associated with off task duration, reducing the likelihood of confounding by these co-exposures.

Other studies examining lower concurrent blood Pb levels, means 1.9 and 3.7 µg/dL, found associations with attention decrements in children ages 8-12 years as assessed by CPT or parent/teacher ratings ([Cho et al., 2010](#); [Nicolescu et al., 2010](#)). Nigg et al. ([2008](#)) did not provide quantitative results but only reported that the association between concurrent blood Pb level (mean 1 µg/dL, detection limit=0.3 µg/dL) and inattention-disorganization in children ages 8-17 years lost statistical significance with adjustment for sex and family income. Despite some evidence for attention decrements at relatively low blood Pb levels, these studies had several limitations in addition to their cross-sectional design. In these older children, higher earlier childhood Pb exposures may have contributed to observed associations. These studies adjusted for parental education but not consistently for other SES factors. They did not consider potential confounding by parental caregiving quality. Further, sufficient information was not reported to assess whether participation was biased to those with higher Pb exposure and attention decrements. Among children ages 8-11 years in Korea, higher concurrent blood Pb level (mean 1.9 µg/dL, detection limit=0.58 µg/dL) was associated with a higher teacher rating of inattention and more commission errors on the CPT but not other parameters of the CPT ([Figure 4-9](#) and [Table 4-11](#)) ([Cho et al., 2010](#)). Urinary cotinine was more strongly associated with CPT performance than blood Pb level and the primary cause of

attenuation of blood Pb effect estimates. Past Pb exposures especially may have had an influence in the study of children ages 8-12 years in Romania, 55% (n = 46/83) of whom lived near a former metal processing plant and had higher concurrent blood Pb levels (mean 5.1 versus 3.2 µg/dL) ([Nicolescu et al., 2010](#)). Among all subjects, a 1 µg/dL increase in concurrent blood Pb level in the 10th to 90th percentile interval (1.8-7.1 µg/dL) was associated with a 5.2% higher (95% CI: -0.02, 13%) teacher rating of inattention and a weaker, imprecise 1.5% (95% CI: -4.4, 8.5) higher parent rating. Blood Pb levels were poorly correlated (r = -0.06, 0.05) with blood levels of aluminum (Al) or Hg, and neither of these other metals was associated with attention ratings. Results did not change substantially in an analysis that excluded the 5 children with blood Pb levels ≥ 10 µg/dL.

Toxicological Studies of Attention

In support of the associations described in the preceding sections for blood Pb level with attention decrements in children, studies have found Pb-induced decreases in attention in animals, although results have not been consistent across studies. Although tests in animals can measure aspects of both attention and impulsivity, behaviors measured with signal detection tests with distraction can be inferred as predominately assessing sustained attention. In this test, animals earn food rewards by responding to a target stimulus and not responding to a distracter light. Poorer sustained attention and greater distractibility are indicated by lack of response to the target and increased response to the distracter light, respectively. The 2006 Pb AQCD ([U.S. EPA, 2006b](#)) reported inconsistent effects of Pb exposure in animals on performance in this test. For example, postweaning Pb exposure that produced blood Pb levels of 16 and 28 µg/dL induced small decreases in attention in adult rats as indicated by small increases in omission and commission errors but only during sessions with long intervals between stimuli ([Brockel and Cory-Slechta, 1999a](#)). Lifetime Pb exposure from birth (blood Pb levels 15, 25 µg/dL) was found to induce distractibility in monkeys at age 9-10 years as indicated by increased responding to irrelevant cues, i.e., distracting stimuli, in a spatial discrimination reversal task. Repeated reversal testing revealed that these deficits likely were not due to sensory or motor impairment ([Gilbert and Rice, 1987](#)).

In animals, Pb-induced decrements in attention have been inferred from tests that were designed to assess attention but that have elicited behaviors that suggest deficits in attention. For example, a study reported that impaired performance on auditory threshold tasks in Pb-exposed monkeys was likely due to lack of attention ([Laughlin et al., 2009](#)). Rhesus monkeys were exposed to Pb acetate from gestation (drinking water of mothers, 3 months prior to mating) to birth or postnatally from birth to age 5.5 months at weaning and had resultant bone Pb levels at 11 years of 7 and 13 µg/g for prenatal and postnatal

groups, respectively, and average blood Pb levels during Pb exposure of 35 and 46 µg/dL, respectively. Animals were tested at age 13 years when blood Pb levels had returned to baseline levels. The inability of some of the monkeys to engage or focus attention on the task at hand yielded fewer available measurements in Pb-exposed animals versus controls. These observations were made in monkeys with higher peak blood Pb levels than those relevant to this ISA.

Epidemiologic Studies of Impulsivity in Children

Compared to attention, measures specific to impulsivity have been examined in few epidemiologic studies of children and mostly in recent cross-sectional studies. However, evidence in children indicates Pb-associated poorer performance on tests of response inhibition which finds coherence with similar evidence in animals. Response inhibition is a measure of impulsivity and in children has been assessed with stop signal tasks, which measures the execution of action in response to stimuli and the inhibition of that action when given a stop signal. Recent cross-sectional studies found that children with higher concurrent blood Pb levels had greater responses with stop signals. Among children ages 8-12 years in Romania, a 1 µg/dL increase in concurrent blood Pb level in the 10th-90th percentile interval 1.8-7.1 µg/dL was associated with a 10% (95% CI: 0, 16) increased false-alarm rate in responses to stop signals with adjustment for city, sex, age, computer experience, handedness, eye problems, number of siblings, parental education, prenatal alcohol and smoking exposure, and parental self report of ever having a psychopathology (Nicolescu et al., 2010). Children in one town lived near a metal processing plant; however, associations were not found with blood Pb levels of Al and Hg. In a case-control study of children with ADHD, Nigg et al. (2008) found that higher concurrent blood Pb level was associated with poorer response inhibition on a stop task

A few studies found associations between higher concurrent tooth (Needleman et al., 1979) or blood (Plusquellec et al., 2010; Nigg et al., 2008) Pb level with higher ratings of impulsivity. Nigg et al. (2008) found associations of concurrent blood Pb levels with parent and teacher ratings of a composite hyperactivity/impulsivity index in a group of children (location not reported) with and without ADHD (ages 8-17 years) with a mean blood Pb level of ~1 µg/dL (detection limit = 0.3 µg/dL). Regression-based path analysis showed that the association between blood Pb level and hyperactivity-impulsivity ratings was mediated by (i.e., occurred through) poorer performance on the more objective stop task. Path analysis produces partial regression coefficients that measure the effect of one variable on another controlling for previous variables. Because hyperactivity and impulsivity are characteristics of ADHD, ratings likely are higher among ADHD cases than controls. If the differential reporting of hyperactivity/impulsivity by ADHD status is unrelated to Pb exposure or blood Pb level, it is less likely to bias associations with Pb.

However, differential rating between cases and controls could produce an artifactual association with blood Pb level if participation by children with ADHD is biased to those with higher Pb exposures and blood Pb levels. Nigg et al. (2008) also used path analysis to show that the Pb-associated increase in hyperactivity/impulsivity was independent of an association with IQ. With adjustment for sex and income, concurrent blood Pb level was directly associated with hyperactivity/impulsivity, and the association was not completely mediated by the blood Pb-IQ association. Instead, the association between blood Pb level and IQ was found to be mediated by the association of Pb with hyperactivity/impulsivity. In a population of Inuit children at ages 5-6 years, the relationship between concurrent blood Pb level and motor function was found to be mediated by (i.e., occur through) the association of blood Pb level with impulsivity (Fraser et al., 2006).

These studies of impulsivity were similar in their adjustment for potential confounding by SES, sex, and parental education (Table 4-11). A few adjusted for smoking exposure. However, parental IQ or caregiving quality were not examined in most studies. Plusquellec et al. (2010) has the most extensive consideration for potential confounding, excluding parental IQ, HOME score, micronutrient levels, Hg, and PCBs (potential co-exposures from high consumption of fish) from final models with blood Pb based on their lack of strong association with impulsivity. There also was no biased participation according to the exposures examined. A limitation of this study is the young age of some children at examination (study range: age 5-6 years). The variable nature of behaviors in younger children may limit the implications of findings.

Toxicological Studies of Impulsivity

The associations described between higher blood Pb level and greater impulsivity in children are supported by findings in animals for Pb-induced increased perseveration and impaired ability to inhibit inappropriate responses. In animals, these effects are supported by studies reviewed in the 1986 and 2006 Pb AQCDs (U.S. EPA, 2006b, 1986b) and recent studies. Animal studies provide more consistent evidence for the effects of Pb exposure on impulsivity in animals than on sustained attention. As mentioned earlier, behaviors displayed by animals in a variety of tests can be identified as predominately reflecting impulsivity. These include tests of Differential Reinforcement of Low Rates of Responding (DRL), FI schedule performance, FI with Extinction, or Fixed Ratio (FR)/waiting-for-reward. Greater impulsivity is indicated by premature responses, decreased pause time between two scheduled events, and increased perseveration.

Behaviors observed in tests of operant conditioning with FI reinforcement schedules also have been used to indicate impaired learning in animals (Section 4.3.2.3), and the

interactions observed between Pb exposure and maternal or offspring stress also may apply to effects on impulsivity. Maternal exposure to 150 ppm Pb with and without stress co-exposure was found to increase overall FI rate and decrease PRP in rats ([Table 4-7](#)). Lifetime (from gestation) Pb exposure resulting in blood Pb levels 11-16 µg/dL increased overall FI rate without stress co-exposure and decreased PRP with stress co-exposure ([Table 4-7](#)) ([Rossi-George et al., 2011](#)). Discrimination reversal learning has been shown to be affected by Pb exposure. In these tasks, an animal is trained to choose between two alternative responses and then is required to reverse that association. Perseveration or lack of inhibition of the original response can be interpreted to involve impulsivity. Spatial and non-spatial discrimination reversal was significantly affected in monkeys after Pb exposure during infancy, after infancy, or continuously from birth, and was exacerbated with distracting stimuli ([Rice, 1990](#); [Rice and Gilbert, 1990b](#); [Gilbert and Rice, 1987](#)). These monkeys had blood Pb levels in the range of 15-36 µg/dL, which includes those relevant to this ISA. Contrary to the findings in monkeys, Hilson and Strupp ([1997](#)), found Pb exposure (Pb acetate in drinking water GD1-PND28, blood Pb levels 26, 51 µg/dL) in rats to slow reversal learning in an olfactory discrimination task. But, analysis of the response patterns showed that Pb exposure shortened the perseverative responding phase of reversal learning and lengthened the post-perseverative phase of chance responding, indicating impairments in associative ability not response inhibition.

The effects of Pb exposure on impulsivity also have been demonstrated as shorter time Pb-exposed animals will wait for reward in FR/waiting for reward testing. In this test, animals can obtain food by pressing a lever a fixed number of times (FR component). Then, free food is delivered at increasingly long time intervals, so long as the animal inhibits additional lever presses. Animals can reset the schedule to return to the FR component at any time. Brockel and Cory-Slechta ([1998](#)) exposed male Long-Evans rats to 0, 50, or 150 ppm Pb acetate in drinking water from weaning, which produced respective blood Pb levels of <5, 11, and 29 µg/dL after 3 months of exposure. After 40 days of exposure, the 150 ppm Pb-exposed rats responded more quickly in the FR component and reset the schedule (thus shortening the waiting period) more often than did the 50 ppm Pb-exposed rats and controls. In the waiting component, average wait time was significantly lower in both Pb exposure groups compared to controls. The rats exposed to 150 ppm Pb also had higher response rates, earned more reinforcers per session, but had a higher response-to reinforcement-ratio than did the 50 ppm Pb group and controls, which indicated less efficient responding. Mechanistic understanding of the Pb-induced effects on responding in the FR/waiting for reward test was provided by a study with similar postweaning exposure to 0, 50, and 150 ppm Pb that yielded respective blood Pb levels of <5, 10, and 26 µg/dL after 3 and 7 months of exposure. Administration of a dopamine (D2) receptor agonist reversed the Pb-induced effects on increasing FR

response rates and decreasing mean waiting time, suggesting a role for dopamine-like receptors in Pb-induced effects on waiting behavior ([Brockel and Cory-Slechta, 1999b](#)).

Pb-induced impulsivity appears to be related to emotionality, which was found in rats trained to perform an olfactory discrimination task, albeit at higher Pb exposures than those relevant to this ISA. Rats were given early postnatal Pb exposure (300 ppm Pb acetate via dam drinking water PND1-PND17 then either 20 or 300 ppm in their own drinking water PND18-PND30), which produced blood Pb levels of 40-60 and 100-140 µg/dL. The rats were tested as young adults on a food-motivated olfactory discrimination task in which rewards for correct responses were occasionally and unpredictably omitted. Pb-exposed animals were more sensitive both to their own errors and to reward omission than controls, suggesting a lowered capacity for regulating arousal and emotion. Administration of the chelator succimer after the Pb exposure period (PND31-PND52) normalized reactivity to reward omission and errors in the Pb-treated rats, but increased reactivity in the control animals ([Beaudin et al., 2007](#)).

The same laboratory using the same Pb exposure protocol also found heightened reactivity to errors in tests of visual discrimination and visual sustained attention ([Stangle et al., 2007](#)). Pb-induced increases in impulsivity were found in rats at PND62 after exposure to 300 ppm Pb (blood Pb level 31 µg/dL at PND52) but not 20 ppm Pb (blood Pb level 13 µg/dL). Impulsivity was inferred from a higher number of premature responses after the basic rules of the task had been learned. Succimer reduced blood Pb level to 3-9 µg/dL at PND52 but did not reduce premature responding at PND62, indicating persistence of the effect. Finally, monkeys exposed to Pb from birth to time of testing at age 3-4 years with blood Pb levels 15 and 25 µg/dL showed slower acquisition of a DRL schedule, which requires an animal to respond at a low rate for rewards. However, the effect was reversible. Pb-exposed monkeys eventually acquired reinforcement rates equal to those of controls ([Rice and Gilbert, 1985](#)).

In summary, several studies in animals indicate that Pb exposure of rodents and non-human primates from birth or after weaning changes behavior in ways consistent with increased impulsivity, primarily as indicated by impaired response inhibition. Some observations of Pb-induced impulsivity in animals were made with blood Pb levels considered relevant for this ISA. The observations for Pb-induced increases in impulsivity in animals provide support for associations found in children of higher blood and tooth Pb levels with lower response inhibition and higher ratings of impulsivity.

Hyperactivity

Studies reviewed in the 2006 Pb AQCD ([U.S. EPA, 2006b](#)) indicated associations between higher concurrent blood Pb level or tooth Pb level and higher parent or teacher ratings of hyperactivity in children ages 6-11 years in the U.S., Asia, and New Zealand ([Rabinowitz et al., 1992](#); [Silva et al., 1988](#); [Gittleman and Eskenazi, 1983](#); [Needleman et al., 1979](#); [David et al., 1976](#)). The case-control or cross-sectional design of studies limited understanding of the temporal sequence between Pb exposure and hyperactivity. Several recent studies, including a prospective study ([Chandramouli et al., 2009](#)), also found associations between blood Pb level and hyperactivity as rated by teachers and parents ([Figure 4-9](#) and [Table 4-11](#)). Overall, studies indicated associations in children ages 3-12 years with mean concurrent blood Pb levels 3.7-12 µg/dL.

The recent prospective study of children in the U.K. helps address some of the limitations of cross-sectional studies by demonstrating an association between higher early childhood (age 30 months) blood Pb level and higher teacher ratings of hyperactivity later in childhood at age 7-8 years and by adjusting for several potential confounding factors, including SES, home facilities score, and family adversity index ([Chandramouli et al., 2009](#)). In addition to the prospective design, the study had high participation rates at baseline and follow-up from a population with similar characteristics as reported in the U.K. census. Increases in hyperactivity were found primarily in the group of children with blood Pb levels >10 µg/dL and were independent of associations with IQ.

The cross-sectional evidence is provided by previous and recent studies conducted in populations with a range of blood Pb levels and with variation in the extent of consideration for potential confounding. A few studies found concurrent blood Pb-associated higher ratings of hyperactivity in populations with mean concurrent blood Pb levels 5-6 µg/dL and examined potential confounding by a large number of factors. Among children ages 5-6 years in Quebec, Canada, Plusquellec et al. ([2010](#)) found that hyperactivity (assessed as observer rating and computer program) was related to concurrent blood Pb level but not HOME score, caretaker IQ or education, or other prevalent exposures in the community including Hg and PCBs. Among children age 7 years in Detroit, MI, hyperactivity was related to concurrent blood Pb levels but not SES, parental education or IQ, or HOME score ([Chiodo et al., 2007](#)). While there is confidence in the internal validity of results from these studies, the applicability to other populations may be limited. The former study included younger children in whom behavioral patterns are less fixed, and the latter study examined a population with high prevalence of prenatal drug exposure.

Other cross-sectional studies found Pb-associated increases in hyperactivity but examined fewer potential confounding factors. Adjustment for SES, maternal education and IQ was

common; however, few adjusted for parental caregiving quality. Associations were found with tooth Pb in children in grades 1-3 ([Rabinowitz et al., 1992](#); [Needleman et al., 1979](#)). Several ([Roy et al., 2009](#); [Chen et al., 2007](#); [Silva et al., 1988](#)) but not all ([Liu et al., 2011b](#)) studies found associations in populations of children ages 3-11 years in the U.S., New Zealand, and Asia with relatively high concurrent blood Pb levels (means 10-12 µg/dL). Nicolescu et al. ([2010](#)) found an association in children ages 8-12 years in Romania with relatively lower concurrent blood Pb levels, median 3.7 µg/dL. Results were adjusted for several potential confounding factors including age, sex, parental education, and prenatal smoking or alcohol exposure. Hyperactivity was not related to Al or Hg, other prevalent exposures in the community. However, potential confounding by other SES variables or parental caregiving quality was not examined.

A recent study did not clearly indicate an effect of Pb on inducing hyperactivity in mice. Pb exposure during gestation and the early postnatal period (PND10) (27 and 109 ppm Pb acetate: ≤ 10 and 42 µg/dL peak blood Pb levels, respectively) increased motor activity of male mice at age 1 year with co-treatment with amphetamines but not female mice ([Leasure et al., 2008](#)). Without amphetamines, Pb induced less activity of mice, and the lower Pb dose inhibited activity more than the higher Pb dose did.

Ratings of Attention Deficit Hyperactivity Disorder-related Behaviors

In addition to finding associations with attention, impulsivity, and hyperactivity, some of the recent epidemiologic studies described in the preceding sections found associations between higher concurrent blood Pb level and higher parental and teacher ratings of ADHD-related behaviors ([Cho et al., 2010](#); [Nicolescu et al., 2010](#); [Froehlich et al., 2009](#); [Roy et al., 2009](#)), calculated as a composite of the various behaviors that are evaluated in the diagnosis of ADHD. The strengths and limitations of these studies have been described in the preceding sections. Main limitations were the cross-sectional design, lack of validation of ADHD ratings with a clinical diagnosis, and lack of consideration for some potentially important confounding factors. Studies considered age, sex, SES or parental education but usually not both, and none considered parental caregiving quality. Thus, the evidence specifically for these total ADHD index ratings were emphasized less than evidence for individual behaviors in drawing conclusions about the effects of Pb exposure on attention, impulsivity, and hyperactivity.

The large, U.S. representative analysis of children participating in NHANES 2001-2004 found an association between concurrent blood Pb level in children ages 8-15 years and parental assessment of child ADHD-related behaviors using the Diagnostic Interview Schedule for Children which uses DSM-IV criteria to identify children at increased risk of meeting diagnostic criteria for ADHD ([Froehlich et al., 2009](#)). Compared with children

with concurrent blood Pb levels <0.8 $\mu\text{g/dL}$ (4% below detection limit of 0.3 $\mu\text{g/dL}$), children with concurrent blood Pb levels >1.3 $\mu\text{g/dL}$ had an elevated odds of parentally-rated ADHD-related behaviors with an OR of 2.3 (95% CI: 1.5, 3.8). These results were adjusted for prenatal and current household smoking exposure, sex, age, race/ethnicity, income to poverty ratio, preschool attendance, maternal age, and birth weight. A similar OR was estimated in analyses restricting the third tertile to children with concurrent blood Pb levels 1.3 - 5.0 $\mu\text{g/dL}$. The strongest association was observed in children with both high blood Pb level and prenatal tobacco smoke exposure. Compared to children with blood Pb levels <0.8 $\mu\text{g/dL}$ and no prenatal exposure to tobacco smoke, children with blood Pb levels >1.3 $\mu\text{g/dL}$ and prenatal exposure to tobacco smoking had the highest odds of parentally-rated ADHD-related behavior (OR: 8.1 [95% CI: 3.5, 18.7]). Although ADHD-related behavior was associated with low concurrent blood Pb levels (1.3 - 5 $\mu\text{g/dL}$), the contribution of higher past Pb exposures of adolescents born in the late 1980s cannot be excluded. Roy et al. (2009) also found an association with teacher ratings of ADHD-related behaviors using DSM criteria in children in Chennai, India; however, the study population included some very young children (i.e., age 3 years) and had relatively high concurrent blood Pb levels (mean: 11.4 $\mu\text{g/dL}$).

Other recent cross-sectional studies found concurrent blood Pb-associated higher ratings of ADHD-related behaviors using instruments that do not follow DSM criteria. Among children ages 8-11 years in Korea, Cho et al. (2010) found a stronger relationship with a total ADHD-related behaviors index as rated by teachers than parents. Mean ADHD ratings by teacher and parents were similar (both 9.1); however, parental ratings had greater variability (SD: 11.5 for parents and 8.6 for teachers), which could have contributed to differences in association. Among children in Romania, concurrent blood Pb level was associated similarly with parent and teacher ratings of ADHD-related behaviors (Nicolescu et al., 2010). As with individual behaviors described in preceding sections, blood Al and Hg levels were not associated with ratings of ADHD-related behaviors. Based on a log-linear model, a 1 $\mu\text{g/dL}$ increase in concurrent blood Pb level within the 10th-90th percentile interval (1.8 - 7.1 $\mu\text{g/dL}$) was associated with a 5.9% increase (95% CI: 0, 13.7) in teacher rating of ADHD-related behavior. The association did not change substantially in an analysis that excluded the 5 children with blood Pb levels ≥ 10 $\mu\text{g/dL}$.

Attention Deficit Hyperactivity Disorder in Children

The 2006 Pb AQCD (U.S. EPA, 2006b) did not review studies of prevalence or incidence of ADHD diagnosis but noted lack of conclusive evidence for the effect of Pb exposure on ADHD based on a few small studies comparing blood or urine Pb levels between children with and without hyperactivity as identified by parents, teachers, or schools

([Gittleman and Eskenazi, 1983](#); [David et al., 1972](#)). As described in the preceding section, several recent cross-sectional studies found associations of concurrent blood Pb level with parent and teacher ratings of a total ADHD index, a composite index of attention, impulsivity, and hyperactivity. Results from a small body of recent studies also indicate associations of higher concurrent blood Pb level with prevalence of diagnosed ADHD in children ages 4-17 years ([Nigg et al., 2010](#); [2008](#); [Wang et al., 2008d](#); [Braun et al., 2006](#)). All of the studies were cross-sectional; thus, the temporal sequence between Pb exposure and ADHD incidence cannot be established. Further, while there are diagnostic guidelines for ADHD, the exact criteria or specific behaviors required can vary. Thus, within studies, there may be variations among subjects in the types of behaviors they displayed that led to a diagnosis of ADHD. While there is coherence with evidence from prospective studies in other populations for associations of Pb biomarker levels with characteristic behaviors, evidence specifically for ADHD prevalence was emphasized less than evidence for individual behaviors in drawing conclusions about the effects of Pb exposure on attention, hyperactivity, and impulsivity.

Associations between concurrent blood Pb level and ADHD prevalence were found in case-control studies conducted in different populations of children. While a limitation of these studies is potential selection bias arising from the nonrandom population sample, a common strength is their independent diagnosis of ADHD in a structured manner using parental and teacher ratings of behavior followed by independent assessment by multiple clinicians using DSM-IV criteria ([Nigg et al., 2010](#); [2008](#); [Wang et al., 2008d](#)). Nigg et al. ([2010](#); [2008](#)) found an association between concurrent blood Pb level and ADHD diagnosis in relatively small (n = 150, 236) groups of children ages 6-17 years from the same community, with controls selected from healthy children who responded to the same mailings, community advertisements, or outreach to local clinics as cases. Wang et al. ([2008d](#)) found an association in a larger (n = 1,260) group of children in China, with controls selected from children admitted as outpatients to the same two pediatric clinics for respiratory infections.

Braun et al. ([2006](#)) found an association in children ages 4-15 years participating in NHANES 1999-2002. ADHD was ascertained by parent-report of ADHD diagnosis or use of stimulant medication, which is subject to reporting bias; however, the examination of multiple risk factors and outcomes in NHANES reduces the likelihood of biased participation and reporting of ADHD by parents of children specifically with higher Pb exposure. NHANES is not a random sample, but a strength over other studies that examined the prevalence of ADHD diagnosis is the large (n = 4,704) sample size and the nationally-representative results produced with adjustment for sampling weights in models. Surveillance data indicate that states with a higher percentage of children with blood Pb levels ≥ 10 $\mu\text{g/dL}$ have lower prevalence of diagnosed ADHD ([CDC, 2012](#),

[2011c](#)). These data do not support the potential for positive confounding of associations observed in the NHANES population by regional differences in blood Pb levels and prevalence of ADHD diagnosis. However, there is the potential for attenuation of associations with blood Pb levels if adjustment is not made for region.

With respect to blood Pb levels associated with ADHD diagnosis, analyses of the concentration-response relationship indicated monotonic increases in ORs across blood Pb level groups ([Wang et al., 2008d](#); [Braun et al., 2006](#)). In the analysis of children in NHANES ages 4-15 years, compared to children with concurrent blood Pb level <0.8 µg/dL (7% below detection limit of 0.3 µg/dL), children with concurrent blood Pb level >2.0 µg/dL (maximum not reported) had higher prevalence of ADHD with an OR of 4.1 (95% CI: 1.2, 14.0). A similar OR was estimated for children with blood Pb levels 2.0-5.0 µg/dL ([Braun et al., 2006](#)). In the study of children in China, the highest OR was found in children ages 4-12 years with concurrent blood Pb levels ≥ 10 µg/dL but also was elevated in the group with blood Pb levels 5-10 µg/dL (OR: 4.92 [95% CI: 3.47, 6.98] compared with children with blood Pb level <5 µg/dL) ([Wang et al., 2008d](#)). Similar to findings in NHANES, Nigg et al. ([2010](#); [2008](#)) found associations at relatively low concurrent blood Pb levels, i.e., population means ~1 µg/dL (1.3%, 0% below detection limit of 0.3 µg/dL). However, because adolescents were examined, higher past Pb exposures could have contributed to the observed associations. Blood Pb levels are higher in early childhood, and among children participating in NHANES who were born 1984-1998, some likely had higher early-life Pb exposures as a results of the use of leaded gasoline in the U.S. ([Braun et al., 2006](#)).

Consideration for potential confounding varied among studies. In three-way analyses of covariance, Nigg et al. ([2008](#)) adjusted for sex and household income, and Nigg et al. ([2010](#)) adjusted for maternal IQ and prenatal smoking exposure. However, in preliminary analyses, Nigg et al. ([2010](#)) considered blood hemoglobin, household income, age, sex, and maternal smoking as potential confounding factors. The analysis of children participating in NHANES adjusted for age, sex, race, prenatal smoking exposure, postnatal smoker in the home, preschool/child care attendance, health insurance coverage, and ferritin levels but initially considered poverty to income ratio, birth weight, and admission to the neonatal intensive care unit ([Braun et al., 2006](#)). The results for children in China were adjusted for similar covariates and also family (parent and sibling) history of ADHD diagnosis, ascertained from clinical records ([Wang et al., 2008d](#)). Family history of ADHD was selected as a covariate based on its association with child ADHD; no information was provided on its association with child blood Pb level. None of the studies of ADHD prevalence considered potential confounding by current parental caregiving quality.

4.3.3.2 Conduct Disorders

Epidemiologic Studies of Conduct Disorders in Children

As described in the introduction to externalizing behaviors, there are two domains of conduct disorders: undersocialized aggressive conduct disorder and socialized aggressive conduct disorder ([as reviewed in Whitcomb and Merrell, 2012](#)). In this evaluation of a relationship with Pb exposure, the two domains are combined because they cannot be easily distinguished in the available epidemiologic studies that combine various behaviors into a composite index, find associations with behaviors within each domain, or examine outcomes characterized by behaviors in each domain. This section also evaluates the evidence for criminal offenses which can be predicted by earlier conduct disorders ([Soderstrom et al., 2004](#); [Babinski et al., 1999](#); [Pajer, 1998](#)).

The 2006 Pb AQCD ([U.S. EPA, 2006b](#)) described several prospective studies that found associations of higher blood, tooth, or bone Pb levels with behaviors related to conduct disorders in children as rated by parents or teachers or criminal offenses in adolescents (also see [Table 4-12](#)). Supporting evidence from recent prospective studies included follow-up of previous cohorts to older ages. Recent cross-sectional studies found associations between concurrent blood Pb level and ratings of behaviors related to conduct disorders, but aside from difficulty in establishing temporality, these recent cross-sectional studies were limited by their less extensive consideration for potential confounding.

Table 4-12 Associations between Pb biomarker levels and behaviors related to conduct disorders in children and young adults.

Study	Study Population and Methodological Details Organized by outcome category. Within each category, results are grouped by strength of study methodology, generalizability ^a	Blood (µg/dL), Tooth, or Bone (µg/g) Pb Metrics Analyzed	Outcomes Analyzed	Effect Estimate (95% CI) ^b
Prospective Studies of Behavioral Ratings				
Dietrich et al. (2001)	186 children followed prenatally (1979-1985) to ages 15-17 yr, Cincinnati, OH Recruitment at prenatal clinic. Moderate follow-up participation but no selective attrition. Mostly African-American. Linear regression model adjusted for HOME, parental IQ, current SES, birth weight. Also considered potential confounding by maternal age, prenatal smoking, alcohol use, and marijuana use, other birth outcomes, ear infections, Fe status, sex, age, caregiver education, public assistance, preschool attendance, # children and adults in home.	0-6 yr avg blood Median (5th-95th): 12 (6.0-26) See Wright et al. (2008)	Self-Report of Delinquent Behavior Score Parental Report of Predelinquent and Delinquent Behavior Score Ages 15-17 yr	0.10 (0.01, 0.19) 0.09 (-0.02, 0.20)
Burns et al. (1999)	322 children followed from birth (1979-1982) to ages 11-13 yr, Port Pirie, Australia. Moderate follow-up participation. Participants had higher birth weight, older mothers, less educated fathers. Log linear regression model adjusted for maternal age, prenatal smoking status, IQ, concurrent psychopathology, and education, birth weight, type of feeding, length of breastfeeding, paternal education and occupation, birth order, family functioning, parental smoking, marital status, HOME score, child IQ.	Lifetime (age 11-13 yr) avg blood GM (5th-95th) Boys 14.3 (13.5-15.1) Girls: 13.9 (13.2-14.6) Intervals analyzed (10th-90th percentiles) Boys: 13.7-14.9 Girls: 13.4-14.5	Aggressive Score, boys Aggressive Score, girls Delinquent score, boys Delinquent score, girls Maternal rating by Child Behavior Checklist at ages 11-13 yr	0.17 (0.08, 0.26) 0.10 (0, 0.21) 0.06 (0.02, 0.09) 0.01 (-0.01, 0.04)

Table 4-12 (Continued): Associations between Pb biomarker levels and behaviors related to conduct disorders in children and young adults.

Study	Study Population and Methodological Details Organized by outcome category. Within each category, results are grouped by strength of study methodology, generalizability ^a	Blood (µg/dL), Tooth, or Bone (µg/g) Pb Metrics Analyzed	Outcomes Analyzed	Effect Estimate (95% CI) ^b
Chandramouli et al. (2009)	488 children followed from age 4 mo (born 1992) to ages 8 yr, Avon, U.K. All births in area eligible. Similar characteristics as U.K. census, high participation at baseline and follow-up. Participants had better educated mothers, who smoked less, better home environment. Log linear regression model adjusted for maternal education and smoking, home ownership, home facilities score, family adversity index, paternal SES, parenting attitudes at 6 mo, child sex. Also considered potential confounding by child IQ.	Age 30 mo blood Mean (SD): NR		ORs for increased score
		Group 1: 0-<2	Antisocial activities	Reference
		Group 2: 2-<5	Parent or teacher rating by Antisocial Behaviour Interview at age 8 yr	0.93 (0.47, 1.83) ^c
		Group 3: 5-<10		1.44 (0.73, 2.84) ^c
		Group 4: >10		2.90 (1.05, 8.03) ^c
Needleman et al. (1996)	212 boys selected from prospective cohort followed from 1st grade to ages 11-14 yr, Pittsburgh, PA Nested case-control. Low follow-up participation. Participants had higher SES, lower maternal IQ, smaller family size, higher IQ, were nonwhite. ANCOVA with behavior scores adjusted for maternal age, IQ, occupation, and education, presence of both parents in home, # children in family, race, history of medical problems, age, score at age 7 yr. Did not consider potential confounding by parental caregiving quality.	Bone at ages 9-14 yr: NR	Delinquent score (square root)	Low Pb: 1.18 High Pb: 1.45, p=0.04
		High bone Pb: upper bound of quintile 3 and higher	Aggressive score (square root)	Low Pb: 2.43 High Pb: 2.98, p=0.009
		Low bone Pb: rest of quintile 3 and lower	Parent rating by Child Behavior Checklist at age 11-14 yr	
		Exact levels NR		
Wasserman et al. (2001)	191 children followed prenatally (1984-1985) to ages 4-5 yr, Pristina, Yugoslavia. Recruitment from prenatal clinics. High follow-up participation, participants had less educated mothers, higher average blood Pb, were Albanian. Log linear regression model adjusted for sex, ethnicity, age, maternal education and smoking history, HOME score, birth weight.	Lifetime (to age 4-5 yr) avg blood	Aggressive Score	Per log increase: 0.08 (-0.19, 0.35)
		Mean of log= 0.86, Mean ~7.2	Delinquent Score	0.32 (0.03, 0.61)
			Maternal rating by Child Behavior Checklist at ages 4-5 yr	

Table 4-12 (Continued): Associations between Pb biomarker levels and behaviors related to conduct disorders in children and young adults.

Study	Study Population and Methodological Details Organized by outcome category. Within each category, results are grouped by strength of study methodology, generalizability ^a	Blood (µg/dL), Tooth, or Bone (µg/g) Pb Metrics Analyzed	Outcomes Analyzed	Effect Estimate (95% CI) ^b
Bellinger et al. (1994b)	1,782 children followed from birth (1979-1980) to age 8 yr, Boston, MA area Recruitment at birth hospital. Moderate follow-up participation. More participants were white, had lower cord blood Pb levels, better birth outcomes. Log linear regression model adjusted for prepregnant weight, race, Cesarean section, maternal marital status, prenatal care, paternal education, colic, child current medication use, sibship size, sex, birth weight. Also considered potential confounding by public assistance, prenatal smoking, maternal education but not parental caregiving quality.	Tooth (age 6 yr) Mean (SD): 3.4 (2.4) Interval analyzed: 1.2-6.3 = 10th-90th percentiles	Total externalizing behavior T score, (inattentive, nervous-overactive, aggressive) Teacher rating by Child Behavior Profile at age 8 yr	0.51 (0.21, 0.81)
Chen et al. (2007)	721 children participating in TLC trial, followed from age 1-3 to 5 yr, Baltimore, MD; Cincinnati, OH; Newark, NJ; Philadelphia, PA. Multi-city, high participation rate. Eligible for chelation trial because of high age 1-3 yr blood Pb levels (20-44 µg/dL). Linear regression-based path analysis adjusted for city, race, sex, language, single parent, parental education and employment, age at blood Pb measurement, caregiver IQ. Considered potential confounding by chelation treatment but not parental caregiving quality. Direct = independent of IQ. Indirect = mediated through IQ.	Age 2 year Mean (SD): 26 (5.1) Interval analyzed: 20-33 =10th-90th percentiles Concurrent Mean (SD): 12 (5.2) Interval analyzed: 6.6 (10th percentile)-10	Oppositional Index Score Direct Indirect Direct Indirect	0.12 (-0.08, 0.31) 0.04 (0.002, 0.07) 0.12 (-0.08, 0.32) 0.05 (-0.03, 0.13)
Cross-sectional studies of Behavioral Ratings				
Braun et al. (2008)	2,867 children ages 8-15 yr (born 1986-1996), U.S. NHANES 2001-2004 Large multi-location study of multiple risk factors and outcomes. Subjects with available data were older, white, higher SES, with lower blood Pb levels, higher birth weight, and fewer had household smokers. Logistic regression model adjusted for child age, sex, and race, poverty income ratio, maternal age, prenatal smoke exposure, cotinine levels. Did not consider potential confounding by parental caregiving quality.	Concurrent blood Q1: 0.2-0.7 Q2: 0.8-1.0 Q3: 1.1-1.4 Q4: 1.5-10 Detection limit = 0.3	Conduct disorder Parental report using Diagnostic Interview Schedule for Children-Caregiver Module at ages 8-15 yr	ORs (yes/no) Q1: Reference Q2: 7.2 (1.1, 49) ^c Q3: 12 (2.4, 65) ^c Q4: 8.6 (1.9, 40) ^c

Table 4-12 (Continued): Associations between Pb biomarker levels and behaviors related to conduct disorders in children and young adults.

Study	Study Population and Methodological Details Organized by outcome category. Within each category, results are grouped by strength of study methodology, generalizability ^a	Blood (µg/dL), Tooth, or Bone (µg/g) Pb Metrics Analyzed	Outcomes Analyzed	Effect Estimate (95% CI) ^b
Chiodo et al. (2007)	452-460 African-American children, age 7 yr (born 1989-1991), Detroit, MI area Recruitment at prenatal clinic. High prevalence prenatal drug exposure. High participation rate. Linear regression model adjusted for sex (both outcomes), caretaker education, HOME, maternal prenatal alcohol use, current marijuana use (delinquent behavior), maternal age, # children in home (social problems). Also considered potential confounding by SES, child age, maternal prenatal and current drug and alcohol use, and IQ, caretaker current psychopathology.	Concurrent blood Mean (SD): 5.0 (3.0) Interval analyzed: 2.1-8.7 = 10th-90th percentiles	Delinquent behavior Social problems Teacher rating by Achenbach Teacher Report Form at age 7 yr	0.09 (0, 0.18) ^d 0.10 (0, 0.20) ^d
Sciarillo et al. (1992)	201 children (born 1984-1987) ages 2-5 yr, Baltimore, MD. Convenience sample. No information on participation rate. 100% African-American. Linear regression adjusted for maternal education, employment status, marital status, current depressive symptom score, # preschool children in the home, child age, sex, and Fe deficiency.	Concurrent blood Mean (SD) Low: 9.2 (2.9) High: 28 (10) Interval analyzed: 5.9 (10th percentile of low group)-10	Total Behavioral Problem score (aggressive, destructive, somatic problems, sleep problems, depressed, social withdrawal, etc) Maternal rating by Child Behavior Checklist at ages 2-5 yr	0.18 (0.04, 0.32)
Nigg et al. (2008)	150 children, ages 8-17 yr, birth yr and location NR. Case-control study of ADHD. Recruitment by community advertisements, mailings, outreach to clinics. Moderate participation rate from eligibles. Could have biased participation by Pb exposure leading to biased behavioral ratings. Did not consider potential confounding factors.	Concurrent blood Mean (SD): 1.03 (0.49) Detection limit=0.3	Oppositional defiant disorder Index Parent, teacher rating Conners Rating Scale-Revised	Pearson r = 0.18, p <0.05
Studies of Criminal Offenses				
Wright et al. (2008)	250 adults followed from birth (1979-1984) to age 19-24 yr, Cincinnati, OH Prospective. Recruitment at prenatal clinic. 90% African-American. Moderate follow-up participation. No selective attrition. Negative binomial regression model adjusted for maternal IQ and education, sex, SES. Also considered potential confounding by maternal prenatal smoking, marijuana use, narcotic use, and prior arrests, HOME score, birth weight, # children in the home, public assistance in childhood.	Age 6 yr blood Pb Median (5th-95th): 6.8 (3.4-18) Age 0-6 yr avg Median (5th-95th): 12 (6.0-26)	Criminal arrests From county records at ages 19-24 yr Age 6 yr Age 0-6 avg	RRs (yes/no): 1.05 (1.01, 1.09) 1.01 (0.98, 1.05)

Table 4-12 (Continued): Associations between Pb biomarker levels and behaviors related to conduct disorders in children and young adults.

Study	Study Population and Methodological Details Organized by outcome category. Within each category, results are grouped by strength of study methodology, generalizability ^a	Blood (µg/dL), Tooth, or Bone (µg/g) Pb Metrics Analyzed	Outcomes Analyzed	Effect Estimate (95% CI) ^b
Fergusson et al. (2008)	853 children followed from birth (1977) to age 21 yr, Christchurch, New Zealand Prospective. High follow-up participation. Participants had lower SES. Negative binomial regression model adjusted for maternal education and prenatal cigarettes/ day, ethnicity, family conflict, physical abuse in childhood. Also considered potential confounding by traffic density in childhood, maternal age, paternal education, average family income, maternal use of punishment, parental drug use, parental bonding, child marijuana use, parent alcohol problems.	Tooth (age 6-8 yr) Mean: 6.2	Number convictions for property or violent offenses From police records at ages 14-21 yr	0.20 (0, 0.40)
Needleman et al. (2002)	340 adolescents, ages 12-18 yr (born 1978-1986), Pittsburgh, PA area. Case-control. 194 cases (county program) and 146 controls (Pittsburgh high schools) from different sources. Low participation from cases. Logistic regression adjusted for race, parental education and occupation, both parents in home, neighborhood crime rate, #children in home. Did not consider potential confounding by parental caregiving quality.	Concurrent Bone Mean (SD) in ppm Cases: 11 (33) Controls: 1.5 (32)	Delinquent status From Juvenile Court records at ages 12-18 yr	ORs (yes/no) for bone Pb level >25 ppm White: 3.8 (1.1, 13) ^c African-American: 2.2 (0.5, 10.0) ^c

^aResults are organized by outcome category, behavior ratings then documented criminal offenses. Within each category, studies are grouped by strength of study design, representativeness of the study population characteristics and Pb biomarker levels examined, and extent of consideration for potential confounding. There is not necessarily a continuum of decreasing strength across studies.

^bUnless otherwise specified, effect estimates are standardized to a 1 µg/dL increase in blood Pb level or 1 µg/g increase in bone or tooth Pb level in the interval from the 10th percentile to 10 µg/dL blood Pb or 10 µg/g tooth or bone Pb or the 90th percentile, whichever is lower.

^cOdds in higher quantile of blood or bone Pb level compared to that in lowest quantile of blood or bone Pb level (reference).

^d95% CI was estimated from a reported p-value of 0.05.

Collectively, the evidence from prospective studies indicated associations of teacher and parental ratings of aggressive, antisocial, and delinquent behavior with biomarkers of cumulative Pb exposure, i.e., age 0-6 year average blood, lifetime (to ages 4-5 or 11-13 years) average blood, tooth, and bone Pb level. Associations with tooth ([Bellinger et al., 1994b](#)) and bone ([Needleman et al., 1996](#)) Pb level, collected prior to or at the same time as behavioral assessments, respectively, were found with adjustment for several potential confounding factors as noted above, with the exception of parental caregiving quality. Collective evidence from prospective studies indicated associations between blood Pb level and ratings of behaviors related to conduct problems with population mean blood Pb levels 6.8-14.3 µg/dL (prenatal maternal, age 6 year, lifetime average to 4-5 or 11-13 years) ([Dietrich et al., 2001](#); [Wasserman et al., 2001](#); [Burns et al., 1999](#)). In a study of children ages 7-8 years in the U.K. born in the 1990s, Chandramouli et al. ([2009](#)) added to the evidence for associations at relatively high blood Pb levels by finding that compared with children ages 8 years with age 30 month blood Pb levels 0-2 µg/dL, children with blood Pb levels >10 µg/dL had increased odds of greater antisocial activities as rated by parents or teachers with an OR of 2.9 (95% CI: 1.1, 8.0). The Boston cohort had lower childhood blood Pb levels ([see Bellinger et al., 1992](#)); however, tooth Pb level was mostly strongly associated with a higher total externalizing behavior score, which also included attention, overactive/nervous, and aggression ([Bellinger et al., 1994b](#)). Weaker associations were found specifically with aggressive or destructive behavior.

As described above, cross-sectional studies also indicated blood Pb-associated higher ratings of conduct disorders. Associations were found with lower blood Pb levels than in prospective studies, concurrent means 1 and 5 µg/dL (ages 7-17 years). However, for these children, the influence of higher Pb exposures earlier in childhood cannot be excluded. Further, because of their various limitations discussed below, cross-sectional results were less of a consideration in drawing conclusions about the effects of Pb exposure on conduct disorders. In the recent analysis of 2,867 children ages 8-15 years participating in NHANES 2001-2004, Braun et al. ([2008](#)) analyzed blood Pb level as a categorical variable and found higher prevalence of conduct disorder as ascertained by parents (Diagnostic Interview Schedule for Children which follows DSM-IV criteria) with concurrent blood Pb levels in the range of 0.8 to 1.0 µg/dL. Compared with children with blood Pb levels 0.2-0.7 µg/dL (6% below detection limit of 0.3 µg/dL), the OR in children with blood Pb levels 0.8-1.0 µg/dL was 7.2 (95% CI: 1.1, 49). The wide 95% CIs could have resulted from the small numbers of cases of conduct disorder. For example, there were 22 children rated as having conduct disorder in the group with blood Pb levels 0.8-1.0 µg/dL. Nigg et al. ([2008](#)) found a blood Pb-associated higher rating (parent or teacher) of oppositional defiant disorder in a population with similarly low concurrent blood Pb levels, means ~1 µg/dL. However, the implications are limited

because of the ADHD case-control design ([Section 4.3.3.1](#)) and lack of consideration of potential confounding factors. Further, because both studies examined adolescents who likely had higher earlier childhood Pb exposures, there is uncertainty regarding the level, timing, frequency, and duration of Pb exposure that contributed to the observed associations.

Consideration for potential confounding varied among the cross-sectional studies of behaviors related to conduct disorders. With the exception of Nigg et al. ([2008](#)), many considered sex, race, SES, and parental education. However, only a few considered parental caregiving quality (i.e., HOME score). An analysis of children in Baltimore, MD adjusted results for child Fe deficiency but examined children ages 2-5 years and analyzed a total behavior problem score, which included various externalizing and internalizing behaviors ([Sciarillo et al., 1992](#)). Chiodo et al. ([2007](#)) found that higher concurrent blood Pb level was associated with higher teacher ratings of social problems and delinquent behavior in children ages 7 years in Detroit, MI with consideration for HOME score. The study population had high prevalence of prenatal drug exposure. Drug exposure did not meet the criteria for inclusion in the model, indicating lack of confounding by this factor. While prenatal drug exposure may not affect internal validity of the results, it may limit the generalizability of the results to the general U.S. population of children. Lack of representativeness also may pertain to the results of Chen et al. ([2007](#)), who examined children participating in a chelation trial because of high blood Pb levels (20-44 µg/dL) at ages 1-3 years.

While few in number, evidence from prospective studies also indicated associations of biomarkers of earlier childhood Pb exposure with delinquent and criminal acts as objectively assessed from government records. These studies of delinquent and criminal acts examined Pb levels in blood or tooth samples collected in the 1980s when Pb exposures were much higher than those of the current U.S. population ([Fergusson et al., 2008](#); [Wright et al., 2008](#)). However, the prospective study design and consideration for several potential confounding factors increase confidence that the observed associations represent a relationship with Pb exposure.

In the Cincinnati cohort, prenatal maternal 1st trimester, age 6 year, and age 0-6 year average blood Pb levels were associated with self- and parent-reported delinquent and antisocial acts at ages 15-17 years ([Dietrich et al., 2001](#)). Wright et al. ([2008](#)) recently extended these findings to include associations with criminal and violent criminal arrests at ages 19-24 years. In models that adjusted for maternal IQ and education, sex, and SES, the relative risks (RRs) for total arrests per 1 µg/dL increment in blood Pb level were 1.07 (95% CI: 1.01, 1.13) for prenatal blood Pb level, 1.01 (95% CI: 0.97, 1.05) for age 0-6 year average blood Pb level, and 1.05 (95% CI: 1.01, 1.09) for blood Pb level at age

6 years. Interactions terms for blood Pb by sex were not statistically significant; however, the attributable risk was considerably higher for males (0.85 arrests/year [95% CI: 0.48, 1.47] for age 6 year blood Pb) than females (0.18 [95% CI: 0.09, 0.33]). A strength of Wright et al. (2008) was the detailed examination of potential confounding by a large number of variables (Table 4-12). All of the examined covariates were weakly correlated with blood Pb levels ($r = 0.24-0.35$), thereby reducing the potential for confounding by the examined factors. Nonetheless, variables such as maternal IQ and education, sex, and SES were included in the model because they were associated with $p \leq 0.05$ with arrests in the full multivariate model or changed the blood Pb level estimate by more than 10%. HOME score was similar between subjects with and without criminal arrest records and did not meet the criteria for inclusion in final models.

The study of the New Zealand cohort also considered several potential confounding factors including several related to SES, family functioning, and parental bonding (Table 4-12) (Fergusson et al., 2008). Per log increase in Pb in teeth obtained between ages 6 and 8 years, there was a 0.49 (95% CI: 0.16, 0.82) increase in the number of documented violent or property convictions at ages 14-21 years. Results were adjusted for SES, ethnicity, parental criminal offenses, and parental alcoholism. The effect estimate for tooth Pb level decreased in adjusted models and was found to account for <1% in the variance of criminal convictions; however, the association remained statistically significant.

The epidemiologic studies described above varied in study design, examination of specific behaviors related to conduct disorders, and method of assessment. The consistency of association of Pb biomarker levels with conduct disorders was corroborated in a recent meta-analysis (Marcus et al., 2010) that included 19 studies (several of which are described above) with a total of 8,561 children and adolescents (range of mean ages: 3.5-18.4 years). Effect estimates were converted to Pearson correlation coefficients, and the combined effect estimate was $r = 0.19$ (95% CI: 0.14, 0.23). The key finding of this study was the robustness of association to many between-study sources of heterogeneity. In the meta-analysis, effect sizes did not significantly differ by population mean blood Pb level, by prospective or cross-sectional design, by the various measures of conduct disorders examined (i.e., oppositional behavior, delinquency, externalizing problem composite measure), or by outcomes assessed using self-report, parent report, teacher report, or criminal records. Adjustment for covariates such as SES, birth weight, parental IQ, and home environment did not attenuate the relationship between Pb biomarker level and conduct disorders. In addition to strengthening the evidence for the independent associations of Pb biomarker levels with conduct disorders, the results indicated that the lack of adjustment for any particular covariate, including HOME score, does not warrant limiting inferences from a particular

study. The major source of heterogeneity in effect estimates was the biomarker of Pb examined. A larger magnitude of effect was estimated for hair Pb levels compared with bone, tooth, or blood Pb levels, which had similar effect sizes. Hair Pb has been purported to be a better indicator of cumulative Pb exposure compared to bone Pb levels due to the high turnover of bone throughout childhood and into adolescence ([Marcus et al., 2010](#)); however, an empirical basis for interpreting hair Pb measurements in terms of Pb body burden or exposure has not been firmly established ([Section 3.3.4.2](#)).

Several studies of conduct disorders aimed to characterize whether associations with Pb biomarkers were independent of effects on cognitive function. These studies found that associations of Pb biomarkers with conduct disorders remained statistically significant in a model that additionally adjusted for child IQ or academic achievement, indicating that Pb exposure may have a direct effect on conduct disorders independent of its effect on cognitive function ([Chandramouli et al., 2009](#); [Fergusson et al., 2008](#); [Burns et al., 1999](#)). However, simple statistical adjustment for cognitive function indices may underestimate an effect of Pb on conduct disorders because a decrement in cognitive function may lie on the causal pathway to behavioral problems. Chen et al. ([2007](#)) used path analysis to characterize the direct effects and indirect effects (mediated/occurring through child IQ) of blood Pb level on an oppositional index at age 5 years and total externalizing behavior ratings at age 7 years. There was evidence of a direct effect of Pb exposure, particularly for total externalizing behavior ratings at age 7 years ([Table 4-12](#)). These findings may have limited applicability to the general U.S. population given that some children in the study had been given chelators at ages 1-3 years because of high blood levels, and it is possible that the observed associations were due to the residual effect of high earlier childhood blood Pb levels (20-44 µg/dL).

Toxicological Studies of Aggression

While recent studies were not identified, evidence available in the 2006 Pb AQCD ([U.S. EPA, 2006b](#)) pointed to the effects of Pb on changes in social behavior of rodents and nonhuman primates. Most observations comprised Pb-induced increases in social and sexual investigation, as indicated by sniffing, grooming, following, mounting, and lordosis behavior. In animals, the social behavior most comparable to findings for conduct disorders in children is aggression; however, the effects of Pb on aggression in animals were inconsistent. In animals, aggression was assessed as threats, attacks, bites, chases, and offensive posture in encounters with other animals, and Pb exposure was found to not affect, decrease, and increase aggression. Pb exposure generally was not found to affect aggression in juvenile animals; however, increased aggression was found in adult animals with high concentration (>2,500 ppm) gestational plus postnatal dietary Pb exposure.

Delville ([1999](#)) found Pb-induced increases in aggression with the lowest concentration Pb exposure examined among all animal studies of aggression. Golden hamsters exposed to 100 ppm Pb acetate GD8-PND42 in drinking water had blood Pb levels of 10 to 15 µg/dL at PND42. As adults at PND45, Pb-exposed animals displayed more aggression as measured by attacking and biting an intruder put in the cage. In mice, higher Pb exposure produced mixed findings. BK:W mice exposed to 1,300 ppm Pb acetate in drinking water from gestation through age 18 weeks displayed increased social and sexual investigation but not aggression (femur Pb level at 34 weeks: 5,364 µM Pb/g ash in males, 4,026 µM Pb/g ash in females) ([Donald et al., 1986](#)). In another study from the same laboratory, BK:W mice were exposed to 2,500 ppm Pb acetate in drinking water from gestation through age 17-18 weeks, and found shorter latencies to aggression in Pb-exposed male mice than in controls ([Donald et al., 1987](#)). In juvenile Long-Evans hooded rats, lactation-only (PND1-PND21) exposure to 670 ppm Pb chloride in drinking water increased “rough and tumble” play behavior at PND26 which was not characterized as aggression because of the lack of injury, submissive posturing, or escape attempts in encounters with other animals ([Holloway and Thor, 1987](#)).

4.3.3.3 Integrated Summary of Externalizing Behaviors

Attention, Impulsivity, and Hyperactivity

Attention, impulsivity, and hyperactivity are included within the attention deficit hyperactivity disorder domain of externalizing behaviors. Although not as extensively as cognitive function, several epidemiologic studies have examined the relationship between Pb exposure in children and attention, impulsivity, and hyperactivity in children and young adults. A majority has examined attention, and some of these studies also examined impulsivity or hyperactivity. Few studies have examined ratings of a composite index of ADHD-related behaviors or ADHD diagnosis and are not the focus of this evaluation. The focus of the evaluation is on evidence for attention but draws on coherence with evidence for impulsivity and hyperactivity, evidence in animals, and that describing potential modes of action. The collective epidemiologic evidence base for attention comprises many prospective studies, which also were reviewed in the 2006 Pb AQCD ([U.S. EPA, 2006b](#)), as well as many cross-sectional studies, some reviewed previously and some recently published. Whether prospective or cross-sectional or previously reviewed or recently published, most of the epidemiologic studies found associations between childhood blood or tooth Pb levels and attention decrements, impulsivity, and hyperactivity ([Figure 4-9](#) and [Table 4-11](#)). Not all results were

statistically significant, but results mostly showed a pattern of attention decrements, impulsivity, and hyperactivity with higher blood or tooth Pb level.

Whether prospective or cross-sectional, most studies had population-based recruitment from prenatal clinics, hospitals at birth, or schools and had moderate to high participation. A few prospective studies had increased loss-to-follow-up in certain groups, for example, lower SES, lower earlier FSIQ, lower HOME score. This potential selection bias can reduce the generalizability of findings to the original study population, but there was not strong indication that participation was biased to those with higher blood Pb levels and greater attention decrements, impulsivity, or hyperactivity. Multiple testing was common; however, in most studies, the consistent pattern of association observed across the ages of blood Pb level and/or behavior examined increases confidence that the evidence is not unduly biased by the increased probability of finding associations by chance alone.

With analysis of prenatal, earlier childhood (e.g., age 6 year) or lifetime average (e.g., to ages 4-5 years or 11-13 years) blood or tooth Pb levels and measures of attention from later in childhood between ages 4 and 20 years, the prospective studies better characterized the temporal sequence between exposure and outcome. In prospective studies, associations of blood or tooth Pb levels with attention were found in diverse populations ages 8-20 years in North America, Australia, and New Zealand. These prospective studies examined populations with higher blood Pb levels (means 7-14 µg/dL) than did cross-sectional studies. Prospective studies found associations of blood and tooth Pb levels with attention as assessed using neuropsychological tests or ratings by parents or teachers. Observations of associations across the various methods of assessment increase confidence that the collective evidence is not unduly influenced by lower attention ratings biased to parents of children with higher blood Pb levels or Pb exposures. Prospective studies that examined attention with the CPT found associations with blood or tooth Pb level. Increases in commission and omission errors or reaction time were associated with higher prenatal (maternal) and earlier childhood (age 3-60 month average, age 78 month) blood Pb levels in the Cincinnati cohort at ages 15-17 years ([Ris et al., 2004](#)) and with higher tooth Pb (from 1st/2nd grade) levels in Boston-area young adults at ages 19-20 years ([Bellinger et al., 1994a](#)). Results from prospective studies also indicated lower parent and teacher ratings of attention in association with higher lifetime average blood Pb levels in children ages 11-13 years in Port Pirie, Australia ([Burns et al., 1999](#)) and with tooth Pb levels (from ages 6-8 years) in children ages 8-13 years in New Zealand, and Boston, MA ([Fergusson et al., 1993](#); [Leviton et al., 1993](#)). The mean blood Pb levels across the populations examined were 6.8 and 8.3 µg/dL for prenatal (maternal or cord) blood, 13.4 for age 3-60 month average, and 14 µg/dL for lifetime average blood. A recent prospective study in the U.K. found that children ages 7-8 years with age 30 month blood Pb levels > 10 µg/dL had higher teacher

ratings of hyperactivity but not selective attention compared with children with blood Pb levels 0-2 µg/dL ([Chandramouli et al., 2009](#)). A null association with attention was found in the Yugoslavia cohort with a mean lifetime (to ages 4-5 years) blood Pb level of 7.2 µg/dL ([Wasserman et al., 2001](#)). However, behaviors may be less reliably measured in the young children ages 4-5 years.

In children, attention was associated with biomarkers of Pb exposure representing several different lifestages and time periods. Prospective studies did not examine a detailed Pb biomarker history, and results do not identify an individual critical lifestage, time period, or duration of Pb exposure associated with attention decrements in children. Associations in prospective studies for attention decrements with tooth Pb level, early childhood average and lifetime average blood Pb levels point to an effect of cumulative Pb exposure. Prospective studies did not examine indicators of more recent Pb exposure. A strength of the prospective studies was their more extensive consideration for potential confounding. Although the specific factors varied by study, prospective studies of attention adjusted for factors such as SES, parental IQ, maternal education, parental caregiving quality, self drug use, prenatal drug and alcohol exposure, and birth outcomes. As described in [Section 4.3.3](#), a few studies have tried to assess potential confounding by parental psychopathology ([Cho et al., 2010](#); [Nicolescu et al., 2010](#); [Silva et al., 1988](#)) but with the use of parental self-report, which may be subject to measurement error. At present, there is not evidence to support parental psychopathology as a direct confounding factor, i.e., contributing to a spurious association with Pb biomarkers when there is none. However, parental psychopathology has been speculated to influence child Pb exposure via a potential relationship with parenting caregiving quality. A clear relationship between parental psychopathology and poorer parenting behavior has not been established ([as reviewed in Johnston et al., 2012](#)), and adjustment for parental psychopathology may control for Pb exposure itself. However, if there is a noncausal correlation between parental psychopathology and Pb exposure and it is mediated by (i.e., occurs through) parental caregiving quality, then direct adjustment for parental caregiving quality may help address potential confounding by parental psychopathology.

Most cross-sectional studies found attention decrements in children ages 3-16 years in association with concurrently measured blood or tooth Pb level. Among studies that examined multiple behaviors, most also found associations with impulsivity and/or hyperactivity. A limitation of cross-sectional associations with concurrent blood Pb levels is the greater uncertainty about the potential for reverse causation. The cross-sectional studies included a mix of previous and recent studies and a wide range of population mean blood Pb levels (1-11.5µg/dL). There also was a mix of preschool-aged and schoolaged children. Among studies of populations with relatively high concurrent blood Pb levels (means 11.1-13.2 µg/dL), most found that higher blood Pb levels were

associated with higher parent or teacher ratings of attention decrements and hyperactivity ([Roy et al., 2009](#); [Kordas et al., 2007](#); [Silva et al., 1988](#)). Higher tooth Pb levels also were associated with higher teacher ratings of hyperactivity in children in grades 1-3 ([Rabinowitz et al., 1992](#); [Needleman et al., 1979](#)). Needleman et al. (1979) also found an association with impulsivity ratings and reaction time. These cross-sectional studies of higher blood Pb levels found associations with adjustment for SES, child age, sex, family structure, and parental education, but most did not examine parental caregiving quality.

Some cross-sectional studies examined lower blood Pb levels (means 1-6.5 µg/dL for ages 4-17 years) closer to those measured in most current U.S. children. There was heterogeneity in the extent of potential confounding and ages of children examined, which makes drawing conclusions difficult. Among the lower blood Pb level studies that had more extensive consideration for potential confounding, most found higher concurrent blood Pb levels to be associated with attention decrements and also with impulsivity or hyperactivity. All of these studies adjusted for or considered potential confounding by SES as well as parental IQ, education, and caregiving quality. In the Rochester cohort, concurrent blood Pb level (mean 6.5 µg/dL) was not associated strongly with attention as rated by study examiners ([Canfield et al., 2003b](#)). However, in the young children ages 4 years, behaviors may be less reliably measured. Also, in the Rochester cohort, the association with attention was attenuated only after adjustment for color/shape knowledge and child IQ. These inter-related outcomes may share some variance. In another study of younger children ages 5-6 years in Canada, higher concurrent blood Pb level was associated with higher examiner ratings of attention decrements, impulsivity, and hyperactivity ([Plusquellec et al., 2010](#)). Among older children (age 7 years) in Detroit, MI, higher concurrent blood Pb level (means: 5.0, 5.4 µg/dL) was associated with higher examiner or teacher ratings of attention decrements and hyperactivity ([Chiodo et al., 2007](#); [2004](#)). These studies also found associations with attention decrements as assessed by the CPT, although not strongly with all examined indices of the CPT. In these populations with high prevalence of prenatal alcohol or drug exposure, results did not indicate confounding by these other exposures. However, the presence of these other exposures may limit the generalizability of results. Further, in older children, the contribution of higher Pb exposures earlier in childhood cannot be excluded. While higher-quality cross-sectional evidence in populations with mean blood Pb levels 5-6.5 µg/dL show associations of blood Pb with attention decrements, impulsivity, and hyperactivity, there are limitations related to young age of subjects or generalizability of the study population.

Other cross-sectional studies examined even lower concurrent blood Pb levels, 1-3.7 µg/dL and found associations with attention, impulsivity, and hyperactivity in children ages 8-17 years ([Cho et al., 2010](#); [Nicolescu et al., 2010](#); [Nigg et al., 2008](#)). These

associations were found with parental or teacher ratings, with attention measured by the CPT, and impulsivity measured with response inhibition tests. Associations between blood Pb level and attention or impulsivity also were found with adjustment for child IQ ([Cho et al., 2010](#); [Nigg et al., 2008](#)), supporting an effect of Pb exposure on these behaviors independent of effects on cognitive function. Despite finding associations with more objective measures of attention or impulsivity, a limitation of these studies was their less consistent consideration for potential confounding factors. Studies varied in adjusting results for SES and parental education, and none considered parental caregiving quality. A limitation specific to Nigg et al. ([2008](#)) is the case-control study design of children with and without ADHD could result in biased ratings of hyperactivity by blood Pb level if participation by children with ADHD is biased to those with higher Pb exposure.

Recent cross-sectional studies also found associations between higher concurrent blood Pb levels and higher parental ratings of a composite index of ADHD-related behaviors, including a large NHANES analysis that used DSM-IV criteria ([Froehlich et al., 2009](#)). In the few available studies, concurrent blood Pb levels were associated with prevalence of diagnosed ADHD in children ([Section 4.3.3.1](#)). There is coherence with evidence from prospective studies for associations of blood and tooth Pb levels with attention, hyperactivity, and impulsivity, which are behaviors evaluated in the diagnosis of ADHD. However, the small number of studies, their cross-sectional or case-control design, inconsistent adjustment for SES and parental education, and lack of consideration for potential confounding by parental caregiving quality preclude conclusions regarding the relationship between Pb exposure and ADHD specifically.

As described above, consideration of potential confounding factors varied particularly among cross-sectional studies. Regardless of study design, adjustment for SES is difficult as it is highly correlated with Pb exposure and there is no single measure that fully represents SES. Residual confounding also is likely by factors not considered or factors measured with error. However, in addition to evidence from prospective studies that considered several well-characterized potential confounding factors, the coherence with evidence that Pb exposure induces greater distractibility and impulsivity in animals increases confidence that the associations of blood and tooth Pb levels with attention decrements and impulsivity observed in children represent a relationship with Pb exposure. Evidence in rodents and monkeys demonstrates Pb-induced impaired response inhibition by decreased interresponse times and increased overall response rates in FI reinforcement schedules ([Table 4-7](#)) ([Rossi-George et al., 2011](#)), slower acquisition of a DRL schedule ([Rice and Gilbert, 1985](#)), and response perseveration on tests of discrimination reversal learning and FR/waiting for reward ([Brockel and Cory-Slechta, 1999b, 1998](#); [Gilbert and Rice, 1987](#)). Coherence is found particularly with evidence in

children for decrements in response inhibition as assessed using the stop signal task. Impulsivity in animals was found with relevant gestational, post-weaning, and lifetime (from gestation or birth) Pb exposures, i.e., producing blood Pb levels 11-29 µg/dL. Pb exposure has not consistently induced attention decrements or distractibility in rats and monkeys ([Brockel and Cory-Slechta, 1999a](#); [Gilbert and Rice, 1987](#)). The findings in children and animals for Pb-associated impulsivity are supported by evidence that Pb affects dopaminergic neurons of the frontal cortex and striatum of the brain by altering dopamine release and receptor density. The circuitry in these regions is thought to mediate response inhibition. Attention decrements, impulsivity, and hyperactivity also have been linked with changes in the hippocampus, and evidence describing the effects of Pb on hippocampal functions further supports the mode of action for the effects of Pb on these behaviors.

Conduct Disorders

As described at the beginning of [Section 4.3.3](#), there are two domains of conduct disorder: undersocialized aggressive conduct disorder and socialized aggressive conduct disorder. Although not examined as extensively as cognitive function or other externalizing behaviors, previous and recent prospective studies consistently demonstrate Pb-associated increases in delinquent behavior, aggression, antisocial activities, and destructive behavior in children as rated by parents and teachers ([Table 4-12](#)). The available evidence does not point to an effect of Pb exposure in one particular domain of conduct disorders. Supporting evidence also is provided by studies showing associations with criminal offenses in adolescents and young adults as assessed with government records. Most studies examined multiple measures of conduct disorders; however, the consistent pattern of association observed across the ages of blood Pb level and/or behaviors examined increases confidence that the evidence is not unduly biased by an increased probability of finding associations by chance alone. Recent cross-sectional studies found associations between concurrent blood Pb level and ratings of conduct disorders, but several had limitations aside from study design ([Nigg et al., 2008](#); [Chen et al., 2007](#); [Chiodo et al., 2007](#)), including limited consideration for potential confounding. A particularly informative cross-sectional study was that finding a 7.2 (95% CI: 1.1, 49) higher odds of parentally-assessed conduct disorder in 2,867 children ages 8-15 years participating in NHANES with concurrent blood Pb levels 0.8-1.0 µg/dL compared with blood Pb levels 0.2-0.7 µg/dL ([Braun et al., 2008](#)). There were 22 children rated as having conduct disorder in the group with blood Pb levels 0.8-1.0 µg/dL, which may have contributed to the wide 95% CI. The association also could have been influenced by higher past Pb exposures of the children. Further, while adjustment was made for age, sex, race, poverty to income ratio, and smoking exposure, potential confounding by

parental caregiving quality was not examined. Evidence of Pb-induced aggression in animals was mixed in adult animals with lifetime Pb exposure beginning in gestation and not indicated in juvenile animals.

The evidence from prospective studies for ratings of behaviors related to conduct disorders in children is substantiated by analyses of school-aged children and young adults (ages 7-17 years) in populations from various locations and SES (i.e., U.K., Cincinnati, Australia) with high participation rates, lack of indication of substantial selection bias, and consideration of several potential confounding factors including multiple SES-related factors, parental caregiving quality, smoking exposure, and birth outcomes ([Chandramouli et al., 2009](#); [Dietrich et al., 2001](#); [Burns et al., 1999](#)). Pb biomarker levels were associated with both parent and teacher ratings of behaviors related to conduct disorders, reducing the likelihood of higher behavioral ratings biased to parents of children with higher Pb biomarker levels. Recent prospective studies of criminal offenses in adolescents and young adults strengthened previous evidence with consideration for potential confounding by factors such as SES, smoking, drug, and alcohol exposure, and parental caregiving quality ([Fergusson et al., 2008](#); [Wright et al., 2008](#)). In the Cincinnati cohort, a 1 µg/dL increase in age 6 year blood Pb level was associated with an increased risk of criminal arrests with an RR of 1.05 (95% CI: 1.01, 1.09) with adjustment for maternal IQ and education, sex, and SES ([Wright et al., 2008](#)). In the New Zealand cohort, a log unit increase in tooth Pb (from ages 6-8 years) was associated with a 0.49 (95% CI: 0.16, 0.82) increase in the number of documented property or violent convictions at ages 14-21 years ([Fergusson et al., 2008](#)) with adjustment for SES, ethnicity, parental criminal offenses, and parental alcoholism. Further support for Pb-associated increases in conduct disorders was provided by a recent meta-analysis that found that the evidence was robust to heterogeneity in study design, definition and assessment method of conduct disorders, potential confounding factors examined, and population mean blood Pb levels ([Marcus et al., 2010](#)). As described in [Section 4.3.3](#), there is not evidence to support parental psychopathology as a direct confounding factor. There hypothetically could be potential confounding by parental psychopathology via a relationship with parental caregiving quality. However, Pb-associated increases in behaviors related to conduct disorders have been found with adjustment for parental caregiving quality.

Associations of measures of conduct disorders at ages 7-24 years (parent/teacher behavior ratings and criminal offenses) with earlier childhood blood (e.g., age 30 month, age 6 year), early childhood average blood (e.g., age 0-6 year), lifetime average blood (to age 11-13 years), tooth, and bone Pb levels pointed to the effects of early childhood or cumulative Pb exposures. Associations were found with a mean lifetime average (to age 11-13 years) blood Pb level of 14 µg/dL, mean age 6 year blood Pb level of 6.8 µg/dL,

mean prenatal maternal blood Pb level of 7.8 µg/dL, and age 30 month blood Pb levels >10 µg/dL. Recent cross-sectional studies found associations with concurrent blood Pb level and lower blood Pb levels, means 1 and 5 µg/dL (ages 7-17 years), but the study limitations detailed above limit strong inferences regarding the effects of Pb exposure on conduct disorders in these populations. Most prospective studies did not analyze Pb biomarker levels at multiple lifestages and time periods, including later childhood and more recent adulthood, or examine differences in association between Pb biomarker levels measured at various lifestages and time periods. The evidence does not identify an individual critical lifestage, time period, or duration of Pb exposure associated with conduct disorders in children or exclude an effect of more recent Pb exposures.

4.3.4 Internalizing Behaviors in Children

4.3.4.1 Epidemiologic Studies of Internalizing Behaviors in Children

Most studies of the effects of Pb on behavior in children have focused on externalizing behaviors such as attention, impulsivity, and conduct disorders. However, several studies also have linked biomarkers of Pb exposure in children with internalizing behaviors characterized by directing feelings and emotions inward, i.e., withdrawn behavior, symptoms of depression, fearfulness, and anxiety. As with externalizing behaviors, internalizing behaviors can be classified into various domains: withdrawn behavior, somatic symptoms, anxiety/depression. Studies examining relationships with Pb exposure examined multiple behaviors and did not clearly indicate that Pb exposure affected a particular domain of internalizing behaviors. Therefore, the evidence for internalizing behaviors is examined as a single group. Some studies found stronger associations for externalizing behaviors than for internalizing behaviors ([Plusquellec et al., 2010](#); [Wasserman et al., 2001](#); [Bellinger et al., 1994a](#); [Sciarillo et al., 1992](#)). Others did not find a clear difference in the strength of association ([Roy et al., 2009](#); [Chiodo et al., 2004](#); [Bellinger et al., 1994b](#)), which is not unexpected since externalizing and internalizing behaviors are positively correlated. Internalizing behaviors frequently were assessed using the Child Behavior Checklist, and as with externalizing behaviors, were rated by parents and/or teachers. Associations with both parent and teacher ratings increase confidence that biased reporting of internalizing behaviors by parents of children with higher blood Pb levels did not unduly influence the collective body of evidence. Most studies had moderate to high follow-up participation. With the exception of the Yugoslavia cohort, participation was not biased to those with higher ratings of internalizing behaviors and higher blood Pb levels. Additionally, in most studies, a

consistent pattern of association was observed across the ages of blood Pb level and/or multiple behaviors examined, which increases confidence that the evidence is not strongly biased by an increased probability of finding associations by chance alone.

The potential for parental psychopathology to influence associations between child Pb and externalizing behaviors was described in [Section 4.3.3](#). Parental psychopathology has the potential to influence associations between child Pb and internalizing behaviors in the same ways since internalizing behaviors also have a familial component. As with externalizing behaviors, available evidence does not support consideration of parental psychopathology as a direct potential confounder of child Pb-internalizing behavior associations. Instead, the ISA evaluates potential confounding by parental caregiving quality (speculated to mediate a potential correlation between parental psychopathology and Pb exposure) and other factors well documented in the literature to be correlated with both Pb exposure and internalizing behaviors such as SES and parental education.

Key evidence was provided by prospective studies in various populations, i.e., Boston, Port Pirie, Australia, and Yugoslavia. Collectively, these studies found higher ratings for internalizing behaviors in children ($n = 322$ - $1,273$, ages 3-13 years) in association with cord blood, concurrent blood (age 3 year), lifetime average blood, and tooth Pb levels ([Wasserman et al., 2001](#); [Burns et al., 1999](#); [Wasserman et al., 1998](#); [Bellinger et al., 1994b](#)). In the Port Pirie cohort, Burns et al. ([1999](#)) found that higher lifetime average blood Pb levels (mean: ~ 14 $\mu\text{g/dL}$) were associated with parental ratings of externalizing behaviors more strongly in boys and with internalizing behaviors (i.e., withdrawn, anxious/depressed, composite) more strongly in girls ages 11-13 years, which may indicate sex differences in the effect of Pb or differences in the types of behaviors that are observed and reported in girls versus boys. Based on a log-linear model, a 1 $\mu\text{g/dL}$ increase in lifetime average blood Pb level was associated with increased odds of an anxious/depressed rating above the median of 1.04 (95% CI: 1.0, 1.09) among 159 boys ages 11-13 years (in the 10th-90th percentile interval of blood Pb level 13.7-14.9 $\mu\text{g/dL}$) and 1.07 (95% CI: 1.01, 1.14) among 163 girls ages 11-13 years (in the 10th-90th percentile interval of blood Pb level 13.3-14.9 $\mu\text{g/dL}$). These associations were found with the adjustment for SES factors, HOME score, family functioning score, and current maternal psychopathology (General Health Questionnaire).

Differences between externalizing and internalizing behaviors also were found in the Yugoslavia cohort but by age of assessment and blood Pb levels. This cohort was examined between ages 3 and 5 years, ages at which behaviors may be less reliably assessed. Among 379 children ages 3 years from the higher and lower Pb exposure towns, higher cord and concurrent blood Pb levels (respective means: 14, 25.8 $\mu\text{g/dL}$) were associated with higher maternal ratings of anxious-depressed, withdrawn, and

externalizing behaviors, with stronger associations found for concurrent blood Pb level ([Wasserman et al., 1998](#)). Among 191 children ages 4-5 years from the lower Pb exposure town, higher lifetime average blood Pb level (mean: 7.2 µg/dL) was associated with higher ratings of delinquent behavior and internalizing behaviors, with stronger associations found for delinquent behavior ([Wasserman et al., 2001](#)). A log increase in higher lifetime average blood Pb level was associated with a 0.22 log higher (95% CI: -0.03, 0.47) rating of withdrawn behavior and 0.19 log higher (95% CI: -0.05, 0.43) rating of anxious-depressed behavior. Results at each age were adjusted for HOME score, sex, ethnicity, and maternal education. Additional covariates included residence type at age 3 years and age, birthweight, and maternal smoking history at ages 4-5 years.

With regard to important lifestages or durations of Pb exposure, results from prospective studies did not clearly demonstrate differences in association among Pb biomarkers measured at various lifestages or time periods. The importance of cumulative Pb exposure was indicated by associations found with lifetime average blood Pb levels in the Port Pirie cohort (to age 11-13 years) and in the Yugoslavia cohort (to age 5 years) and with tooth Pb (from age 6 years) levels in Boston children at age 8 years. In Boston children, Pb levels measured in teeth (mean: 3.4 µg/g) but not cord blood were associated with a teacher rating of internalizing behavior score > 60 at age 8 years ([Bellinger et al., 1994b](#)). In another Boston-area cohort, tooth (collected at first or second grade) Pb levels were not associated with self-rated symptoms of depression (Profile of Mood States questionnaire) at ages 19-20 years ([Bellinger et al., 1994a](#)). Prospective studies did not analyze a detailed history of Pb biomarker levels to evaluate persistence of effects of early exposure or to identify an individual critical lifestage or time period of Pb exposure associated with increases in internalizing behaviors. The available evidence does not preclude an effect of later childhood or more recent Pb exposure.

In the Cincinnati cohort, Dietrich et al. ([1987](#)) with structural equations showed that associations of prenatal maternal and age 10 day blood Pb level (respective means: 8.3, 4.9 µg/dL) with poorer mood in infants ages 6 months (n = 185) were indirect, meaning they were mediated through associations of Pb with shorter gestation or lower birth weight. These results suggest that Pb may exert its effects by impairing nervous system development. The fetal period is an active period of neuronal differentiation, dendritic branching, and synaptogenesis, which if impaired by Pb exposure, could have broad implications on subsequent neurodevelopment. There are few such analyses, and the implications are limited by the lower reliability of mood assessed in infancy.

Cross-sectional studies found associations between concurrent blood ([Liu et al., 2011b](#); [Roy et al., 2009](#)) or hair ([Bao et al., 2009](#)) Pb levels and teacher and parent ratings of internalizing behaviors in children in China and India (n = 303-756, ages 3-16 years).

Mean concurrent blood Pb levels in children ages 3-7 years were 11 and 14 µg/dL. Results were adjusted for family income and parental education but not caregiving quality. In the few studies of populations with mean blood Pb levels ~5 µg/dL, results were inconsistent. Chiodo et al. ([2004](#)) found an association with internalizing behaviors with adjustment for SES, maternal hard drug use, and marital status in 175 children age 7.5 years in Detroit, MI who had high prevalence of prenatal alcohol exposure. HOME score, maternal education, and prenatal alcohol and drug exposure were not found to influence associations with blood Pb level. While prenatal alcohol and drug exposure may not affect the internal validity of results, they may limit generalizability of the results to the general population of U.S. children. A study that examined 79-91 Inuit children (age 5 years) in Quebec, Canada with Hg and PCB co-exposures from a high fish diet, did not find statistically significant associations between concurrent blood Pb level and internalizing behaviors with consideration of potential confounding by parental caregiving quality, caregiver education and IQ, blood Hg or PCB levels, and prenatal smoking and alcohol exposure ([Plusquellec et al., 2010](#)).

Associations of Pb biomarkers with internalizing behaviors in children were observed with consideration for a wide range of potential confounding factors, most commonly, age, birth outcomes, parental education, and other SES-related factors. Parental caregiving quality was evaluated in few studies. Blood Pb-associated higher ratings of internalizing behaviors were found with adjustment for HOME score in the Yugoslavia cohort ([Wasserman et al., 2001](#); [1998](#)), and HOME, family functioning, and current maternal psychopathology (General Health Questionnaire) in the Port Pirie cohort ([Burns et al., 1999](#)). Several studies, each of which adjusted for a different set of covariates, found slightly attenuated or larger effect estimates in multivariate models than unadjusted models ([Wasserman et al., 2001](#); [Burns et al., 1999](#); [Bellinger et al., 1994b](#)). Collectively, these observations increase confidence that the associations observed with Pb biomarkers reflect a relationship with Pb exposure.

4.3.4.2 Toxicological Studies of Internalizing Behaviors

As in epidemiologic studies, toxicological studies have focused more on cognitive function and externalizing behaviors and less on internalizing behaviors. The associations described in the preceding section for Pb biomarker levels with ratings of withdrawn behavior and symptoms of depression and anxiety in children are supported by findings of Pb-induced anxiety, emotionality and depression-like behaviors in animals.

Emotionality has been indicated by increased disruption in performance and frustration in response to errors and reward omission in visual or olfactory discrimination task trials in

Pb-exposed rats ([Beaudin et al., 2007](#); [Stangle et al., 2007](#)). In each study, Long-Evans rats were exposed to Pb during and after lactation (300 ppm Pb acetate via dam drinking water PND1-PND17 then either 20 or 300 ppm PND17-PND30 in own water, with respective blood Pb levels of 13 and 31 µg/dL on PND52). However, this exposure regimen in mice was reported to produce higher peak blood Pb levels at the end of exposure, i.e., 40-60 or 100-140 µg/dL. In Beaudin et al. ([2007](#)), greater disruption in performance (i.e., failure to enter testing alcove) after committing errors and having rewards omitted was found in rats with blood Pb levels 13 and 31 µg/dL tested at age 9-15 weeks. Pb-exposed rats also had greater response latency with reward omission as indicated by the entrance into the testing alcove but failure to respond within a set period of time. In Stangle et al. ([2007](#)), increased reactivity to errors and reward omission was found in rats with blood Pb levels 31 µg/dL. In rhesus monkeys, emotional dysregulation was indicated by tactile defensiveness after exposure to Pb acetate/50% glucose in 4 cc daily milk formula from PND8 to ages 1-2 years to produce target blood Pb levels 35-40 µg/dL ([Moore et al., 2008](#)).

In other studies, rats showed emotionality and depression-like behavior in the open field test or forced swim test (i.e., Porsolt's Test) with Pb exposure. The open field test monitors activity levels and movements of animals in three dimensions. Depression-like behavior is indicated by freezing behavior and low levels of activity. Emotionality is indicated by grooming or freezing. In the forced swim test, animals are placed in a vertical cylinder of water from which there is no escape and monitored for duration of struggling or attempt to escape. Animals that stop quickly are ascribed a depression-like phenotype. As for many other neurobehavioral responses, sex-specific differences were found. Pb-exposed male Wistar rats showed increased emotionality in the open field test as indicated by fewer counts of rearing and longer time freezing. Pb-exposed females showed a depression-like phenotype in the forced swim test as indicated by longer time of immobility ([de Souza Lisboa et al., 2005](#)). While measured blood Pb levels of rats were low, 5-7 µg/dL, they were measured after a lag in exposure (PND70) and produced by oral gavage (10 mg/day to dams), a route that may be less relevant to human routes of Pb exposure. Pb-induced immobility in the forced swim test also was found with 6-week postnatal (PND30-PND72) Pb acetate exposure via drinking water but producing a mean blood Pb level of 40 µg/dL 6-7 weeks after exposure ceased. Reducing internal Pb dose with the chelator succimer reversed Pb-induced immobility ([Stewart et al., 1996](#)).

Depression initially may seem like an unexpected effect of immune modulation, but it has been linked to an interaction between the CNS and the immune system via alterations in cytokines such as IL-6 ([Section 4.6.6.1](#)). Dyatlov and Lawrence ([2002](#)) found that dietary Pb exposure through lactation and a brief period after weaning (500 µM, PND1-PND22, resultant blood Pb level: 17 µg/dL) potentiated sickness behavior in mice in response to

bacterial infection. Sickness behavior was evidenced by an increase in serum IL-6 levels with an accompanying decrease in food and water intake and decrease in body weight gain. This phenotype was correlated with decreases in the populations of several T cell subtypes. Pb exposure also potentiated release of IL-1 β , which plays an important role in inflammatory responses to infection and has been shown to inhibit hippocampal glutamate release in young but not aged animals. Sickness behavior also was induced in Pb-exposed animals with IL-6 and IL-1 administration without infection, further supporting a role for immunomodulation in mediating sickness behavior.

Pb exposure had mixed effects on anxiety-related responses as measured by the elevated plus maze, which assesses behavior of rodents in an unfamiliar environment. The maze is elevated above the floor and consists of two arms that are enclosed with walls intersected with two arms that have no walls. The animal is placed in the center of the maze, and longer latency to enter an open arm, and lower frequency and duration of entries into an open arm are indicative of anxiety. Postnatal (PND1-PND30) exposure of female rats to 2,000 ppm Pb acetate in drinking water, yielding blood Pb levels 34 $\mu\text{g/dL}$, induced an increase in anxiety-related behavior at PND60, as indicated by a lower percentage of open arm entries and less time spent in the open arms ([Fox et al., 2010](#)). In a study of gestational/lactational (GD1-PND24) exposure to 2.84 mg/mL Pb acetate trihydrate in drinking water, Pb-exposed Sprague-Dawley rats did not differ in anxiety-related responses from controls. Blood Pb levels of these Pb-exposed rats were higher than those relevant to this ISA, 69.8 $\mu\text{g/dL}$ at PND25 ([Molina et al., 2011](#)).

4.3.4.3 Summary of Internalizing Behaviors

Internalizing behaviors, i.e., withdrawn behavior, symptoms of depression and anxiety, have been examined less than externalizing behaviors (e.g., attention, conduct disorders). However, several prospective studies found associations of higher parental and teacher ratings of internalizing behaviors with higher tooth or lifetime average blood Pb levels in children ages 8-13 years ([Burns et al., 1999](#); [Bellinger et al., 1994b](#)) and higher cord, concurrent, and lifetime average blood Pb levels in children ages 3-5 years ([Wasserman et al., 2001](#); [1998](#)). Collectively, the lack of biased participation by subjects with higher blood Pb levels and associations found with both parent and teacher ratings increase confidence that the evidence is not unduly influenced by biased reporting of behaviors by parents of children with higher blood Pb levels. The prospective studies found associations with adjustment for several potential confounding factors, including age, birth outcomes, SES, parental education, and parental caregiving quality. As described in [Section 4.3.3](#), there is not evidence to support consideration of parental psychopathology as a direct confounding factor. There hypothetically could be potential confounding by

parental psychopathology via a relationship with parental caregiving quality. However, Pb-associated increases in internalizing behaviors have been found with adjustment for parental caregiving quality. In prospective studies, associations were found with tooth Pb levels and lifetime average, cord, and concurrent blood Pb levels. While, there is not a clear indication of an individual critical lifestage or time period of Pb exposure associated with internalizing behaviors, several observations point to an effect of cumulative Pb exposure. These results do not preclude an effect of later childhood or more recent Pb exposures. In prospective studies, associations with internalizing behaviors were found with mean lifetime average blood Pb levels of 14 µg/dL (to age 11-13 years) ([Burns et al., 1999](#)) and 7.2 µg/dL (to age 4.5-5 years) ([Wasserman et al., 2001](#)). In the Yugoslavia cohort at ages 4-5 years, in whom behaviors may be less reliably rated, lifetime average blood Pb level was associated more strongly with the rating of delinquent behavior than ratings of internalizing behaviors ([Wasserman et al., 2001](#)). Cross-sectional studies found concurrent blood Pb-associated increases in internalizing behaviors in children ages 3-16 years. Studies that considered potential confounding by a similar set of factors as the prospective studies examined children ages 5-7 years with mean concurrent blood Pb levels ~5 µg/dL and found inconsistent associations ([Plusquellec et al., 2010](#); [Chiodo et al., 2004](#)).

Evidence in children is supported by observations that dietary Pb exposure resulted in depression-like behavior and emotionality in rodents (gestational-lactational, PND1 to PND 22 or 30, post-lactation) and rhesus monkeys (neonatal exposure to age 1-2 years) ([Moore et al., 2008](#)), with some evidence in rodents at blood Pb levels relevant to this ISA (13, 17 µg/dL) ([Beaudin et al., 2007](#); [Dyatlov and Lawrence, 2002](#)). Evidence for Pb-induced anxiety in animals is mixed. Mode of action support is provided by well-documented evidence for Pb-induced changes in the HPA axis ([Section 4.3.2.3](#)) and dopaminergic and GABAergic systems ([Sections 4.2.2.2, 4.3.10.4, and 4.3.10.8](#)), which are involved in regulating mood and emotional state.

4.3.5 Psychopathological Effects in Adults

4.3.5.1 Epidemiologic Studies of Psychopathological Effects in Adults

The potential effects of Pb exposure on psychopathological effects (e.g., anxiety, depression, schizophrenia) have been examined less so in adults than in children and less than cognitive function in adults. Most studies of Pb and psychopathologies in adults have examined mood states, which are an integral part of the neurocognitive test battery

of the World Health Organization (WHO). There is evidence that indices of the Profile of Mood States may be particularly sensitive to toxicant exposures ([Johnson et al., 1987](#)). As with other nervous system endpoints in adults, evidence reviewed in the 2006 Pb AQCD ([U.S. EPA, 2006b](#)) pointed to effects in Pb-exposed workers. Higher prevalence of self-reported symptoms of depression, anxiety, and tension was found among Pb-exposed workers (n = 43-576, mean concurrent or peak blood Pb levels: 31-79 µg/dL) in association with higher blood Pb levels or compared with unexposed controls (n = 24-181, mean blood Pb levels: 15-38 µg/dL) ([Schwartz et al., 2005](#); [Maizlish et al., 1995](#); [Parkinson et al., 1986](#); [Baker et al., 1985](#); [Baker et al., 1984](#); [Lilis et al., 1977](#)). Several studies considered potential confounding by age, sex, and education. Medical conditions, smoking, and alcohol use were examined in one or two studies. Only Maizlish et al. ([1995](#)) examined other occupational exposures (previous occupational exposure to solvents). Occupational Mn exposure has been linked with anxiety and depression but was not examined as a potential confounder of Pb associations. Most studies were cross-sectional, which makes less certain the temporal sequence between Pb exposure and development of psychopathological effects.

The few studies of adults without occupational Pb exposures participating in NAS and NHANES demonstrated associations of concurrent blood and bone Pb level with symptoms of depression and anxiety. As bone Pb is a major contributor to blood Pb levels in adults without current occupational Pb exposure, cross-sectional associations for both blood and bone Pb may indicate effects of cumulative Pb exposure. Although specific covariates varied among studies, these previous and recent cross-sectional studies found associations with adjustment for several potential confounding factors, including age, education, smoking, alcohol use, employment status, and SES. Further, the examination of multiple exposures and outcomes in these studies reduces the likelihood of participation conditional specifically on Pb exposure and psychopathological effects.

Analyses of 526 men ages 48-70 years in the NAS indicated associations of both higher concurrent blood (mean: 6.3 [SD: 4.2] µg/dL), patella (mean: 32.1 [SD: 19.8] µg/g), and tibia (mean: 21.9 [SD: 13.5] µg/g) Pb levels with higher prevalence of a combined index of self-reported depression and anxiety ([Rhodes et al., 2003](#)). In a recent analysis of 744 NAS men ages 48-94 years, Rajan et al. ([2007](#)) found associations of symptoms assessed using the Brief Symptom Inventory with patella and tibia Pb levels (measured within 1.3 years of outcomes). A 14 µg/g increase in tibia Pb level was associated with an increased odds of an anxiety score one standard deviation above the mean of 1.18 (95% CI: 0.98, 1.42) and of depression score above the median of 1.16 (95% CI: 0.97, 1.38). Similar effect estimates were found for patella Pb level. Effect modification by ALAD genotype was not in a consistent direction. For most of the evaluated mood symptoms, ORs for tibia bone Pb levels were larger among the 587 men with the ALAD 1-1 genotype. In

contrast, ORs for associations between patella Pb levels and symptoms such as depression and positive symptom distress index were larger among the 119 ALAD-2 carriers. In the NAS, effect modification by ALAD genotype also was inconsistent for associations between tibia Pb levels and cognitive performance ([Rajan et al., 2008](#)) ([Section 4.3.2.7](#)). The relationship between ALAD-2 genotype and Pb bioavailability is not clear ([Section 4.2.3.3](#)).

A recent analysis of 1,987 adults ages 20-39 years participating in NHANES 1999-2004 was the largest study of psychopathological effects in adults and included both men and women of multiple races and ethnicities ([Bouchard et al., 2009](#)). However, only concurrent blood Pb levels were available for analysis. Major depressive disorder, panic disorder, and generalized anxiety disorder were examined using the WHO Composite International Diagnostic Interview, which follows DSM criteria. Adults with concurrent blood Pb levels >0.7 µg/dL (detection limit = 0.2, 0.3 µg/dL) had higher prevalence of all three self-reported disorders. Adults in the highest quintile of concurrent blood Pb level (≥ 2.11 µg/dL) had the highest OR for major depressive disorder (OR: 2.3 [95% CI: 1.1, 4.8]) and panic disorder (OR: 4.9 [95% CI: 1.3, 18]) compared with nonsmoking adults with blood Pb levels 0.3-0.7 µg/dL with adjustment for age, sex, race, education, and poverty to income ratio. A monotonic increase in ORs was not found across the quintiles of blood Pb levels. For all endpoints, ORs were larger in analyses excluding current smokers. While associations were found with relatively low concurrent blood Pb levels, there is uncertainty regarding the magnitude, timing, frequency, and duration of Pb exposure that contributed to the observed associations.

In analyses of small cohorts in California and New England born 1959-1966, Opler et al. ([2008](#); [2004](#)) reported associations between higher levels of cord plasma δ -ALA and subsequent diagnosis of schizophrenia spectrum disorder (ascertained from records and structured interview) in adolescents and adults. Because of the lack of direct measurements of Pb biomarker levels, post hoc analysis, and limited consideration for potential confounding, conclusions regarding a relationship between schizophrenia and Pb and are not warranted. Investigators measured δ -ALA levels in stored serum samples as surrogates for Pb exposure only citing previous observations of a high agreement (90%) between samples with δ -ALA levels ≥ 9.05 ng/mL and blood Pb levels ≥ 15 µg/dL. In the California cohort, δ -ALA level ≥ 9.05 ng/mL was associated with schizophrenia spectrum disorder with an OR of 2.43 (95% CI: 0.99, 5.96), adjusted for maternal age at delivery. In pooled analyses of the California and New England cohorts (n = 200), δ -ALA level ≥ 9.05 ng/mL was associated with schizophrenia spectrum disorder with an OR of 1.92 (95% CI: 1.05, 3.52), with adjustment for maternal age and education. An adjusted OR was not presented for the New England cohort alone, and it

appeared that the association in the pooled dataset was influenced more by that found in the California cohort.

4.3.5.2 Toxicological Studies of Mechanisms for Schizophrenia

An environmental origin of schizophrenia was proposed years ago ([Tsuang, 2000](#)), and while epidemiologic evidence is inconclusive, toxicological studies have found Pb exposure to induce changes that have been associated with schizophrenia-related effects ([Figure 4-10](#)). Pb exposure has been shown to reduce function in the NMDA receptor (NMDAR) and decrease hippocampal neurogenesis. Pb may bind a divalent cation site in the NMDAR and allosterically inhibit glycine binding ([Hashemzadeh-Gargari and Guilarte, 1999](#)). Antagonists of the NMDAR glycine site have been shown to exacerbate schizophrenia symptoms in affected individuals and induce a schizophrenic phenotype in unaffected subjects ([Coyle and Tsai, 2004](#)). Developmental Pb exposure inhibits neurogenesis in animal models ([Section 4.3.10.9](#)). Evidence indirectly suggests that a decrease in hippocampal degenerate gyrus (DG) neurogenesis could be a mode of action for Pb-associated schizophrenia induction. Decreased neurogenesis is seen in patients with schizophrenia ([Kempermann et al., 2008](#); [Reif et al., 2006](#)) and animal models of schizophrenia ([Maeda et al., 2007](#)), and clozapine, a treatment for schizophrenia, restores hippocampal DG neurogenesis in animal models of schizophrenia ([Maeda et al., 2007](#)) ([Figure 4-10](#)). These DG pathways are also NMDAR-dependent.

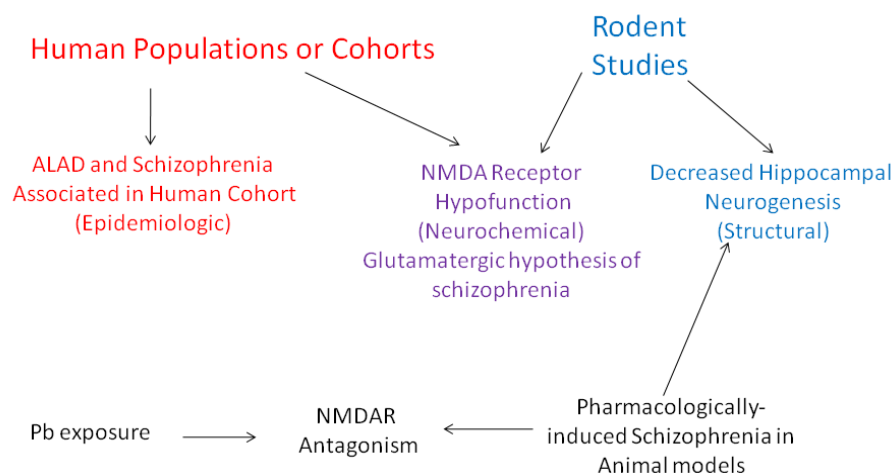


Figure 4-10 Hypothetical representation of the contribution of Pb exposure to the development of a phenotype consistent with schizophrenia.

4.3.5.3 Summary of Psychopathological Effects in Adults

Studies of Pb exposure and behavior in adults have focused on mental disorders rather than the externalizing behaviors that have been examined in children. Evidence links occupational Pb exposure with self-reported increases in symptoms of depression and anxiety, although the cross-sectional design and potential confounding by other occupational exposures such as Mn limit the implications of findings from these occupational studies. However, supporting evidence is provided by a few but large ($n = 744$ and $1,787$) cross-sectional studies in nonoccupationally-exposed adults that found associations of concurrent blood ([Bouchard et al., 2009](#)) and bone ([Rajan et al., 2007](#)) Pb levels with symptoms of depression and anxiety as assessed with widely-used structured questionnaires such as the Brief Symptom Inventory and the WHO Composite International Diagnostic Interview. Evidence was provided by the NAS study of men (primarily white) and study of men and women (various races/ethnicities) participating in NHANES, both of which involve the examination of multiple exposures and outcomes. Although specific factors varied among studies, studies in adults with and without occupational Pb exposure found associations with adjustment for several confounding factors, including age, sex, and education, and in a few studies, smoking and alcohol use. The cross-sectional nature of these studies makes uncertain the temporal sequence between Pb exposure and development of psychopathological effects and the critical level, timing, frequency, and duration of Pb exposure. Both blood and bone Pb levels in

adults reflect cumulative exposure, and it is uncertain what are the relative contributions of past versus recent Pb exposures to the observed associations. The epidemiologic evidence is supported by observations that Pb exposure (gestational-lactational, lactational, postlactational) induces depression-like and emotional phenotypes in animals ([Section 4.3.4.2](#)). The mode of action is supported by evidence for Pb-induced changes in the HPA axis and dopaminergic and GABAergic CNS processes, which are involved in regulating mood and emotional state.

An epidemiologic study found a cord plasma δ -ALA-schizophrenia association but was insufficiently informative because of the use of an indirect biomarker of Pb exposure. However, toxicological studies have shown that Pb exposure decreases NMDAR function and hippocampal DG neurogenesis, which are found in animal models of schizophrenia (agitation, trouble finding food, reduced swimming behavior).

Epidemiologic evidence also indicates associations of Pb biomarker levels with symptoms of depression and anxiety in children and adults as rated by self, parents, or teachers ([Section 4.3.4.1](#)). Differences between children and adults for a relationship between Pb and other behaviors may be related to differences in what endpoints are examined. Several studies in children and young adults have examined attention and conduct disorders; studies of older adults did not examine such behaviors. Differential effects in children and adults also may be expected given the predominance of different neurophysiological processes operating at different ages, for example, neurogenesis and brain development in children and neurodegeneration in adults. Differences in the effects of Pb exposure between children and adults also may be related to differences in Pb exposure profiles by age.

4.3.6 Sensory Organ Function

4.3.6.1 Auditory Function

Epidemiologic Studies of Auditory Function in Children

Although not as widely examined as cognitive and behavioral outcomes, several studies found associations of higher blood Pb level with higher hearing thresholds or poorer auditory processing in children, with evidence limited largely to that described in the 2006 Pb AQCD ([U.S. EPA, 2006b](#)). The prospective Cincinnati study with repeat measures of blood Pb prenatally to age 5 years provided information on the temporal sequence between Pb exposure and auditory effects and potential critical time periods of

Pb exposure and had extensive consideration for potential confounding. In 215 Cincinnati subjects at age 57 months, poorer auditory processing was associated with higher prenatal maternal, neonatal (age 10-day), yearly age 1 to 5 year (means: 10.6-17.2 µg/dL), and lifetime average blood Pb levels, with the strongest associations found for neonatal blood Pb level (mean: 4.8 [SD: 3.3] µg/dL) ([Dietrich et al., 1992](#)). A 1 µg/dL higher neonatal blood Pb level was associated with a 0.26-point ($p \leq 0.10$) and 0.20-point ($p \leq 0.01$) lower score on the total and left ear Filtered Word test (indicating incorrectly identified, filtered, or muffled words), respectively, with adjustment for hearing screen, social class, HOME score, birth weight, gestational age, obstetrical complications, and maternal alcohol consumption. Overall, the findings pointed to a stronger effect of Pb exposure in infancy.

Additional support was provided by the large U.S. cross-sectional NHANES II (n = 4,519) ([Schwartz and Otto, 1987](#)) and Hispanic Health and Nutrition Examination Survey (HHANES, n = 3,262) ([Schwartz and Otto, 1991](#)) studies. The examination of multiple exposures and outcomes in these studies reduces the likelihood of participation conditional specifically on Pb exposure and hearing function. Each study found an association between higher concurrent blood Pb level and higher hearing thresholds in children (ages 4-19 years). In HHANES, an increase in concurrent blood Pb level (median: 8 µg/dL) from 6 to 18 µg/dL also was associated with a 15% increase in the percentage of children with a substandard hearing threshold at 2,000 Hz. Higher concurrent blood Pb level also was associated with higher hearing thresholds across several frequencies in a smaller (n = 155) study of similarly aged (4-14 years) children in Poland with similar blood Pb levels (median: 7.2 µg/dL [range: 1.9-28 µg/dL]) ([Osman et al., 1999](#)). In each of the studies in children, associations persisted with concurrent blood Pb levels <10 µg/dL. Each of these studies adjusted for different potential confounding factors, but in stepwise regression analyses, most considered parental education, SES, colds, and nutritional factors. Across studies, associations were found with adjustment for factors such as age, sex, ethnicity, family income, concurrent or past colds, antibiotic use, degree of urbanization, and Apgar score (assessment of newborn overall well-being).

Mechanistic support for associations with higher hearing thresholds in children was provided by a few studies that found associations of blood Pb level with lower brainstem auditory evoked potentials in children. Brainstem auditory evoked potentials measure nerve electrical activity and are indicators of neurological auditory function. In prospective analyses of the Mexico City cohort, decreased brainstem auditory evoked potentials were associated with prenatal blood Pb levels at age 9 days (n = 30) ([Rothenberg et al., 1994b](#)) and ages 5-7 years and with postnatal blood Pb levels at ages 5-7 years (n = 100-113) ([Rothenberg et al., 2000](#)). At age 5-7 years, the shape of the concentration-response relationship differed between prenatal maternal and postnatal

(ages 1 and 4 years) blood Pb level. Linear associations were found for age 1 year and age 4 year blood Pb level (means reported for age 18 and 60 months: 10.8 and 8.0 µg/dL, respectively). Prenatal maternal blood Pb levels showed a biphasic relationship, with lower evoked potentials found with increasing blood Pb levels 1-8 µg/dL and higher evoked potentials found with increasing blood Pb levels 8-30 µg/dL. Results were adjusted for age, sex, and head circumference. Associations with increased latencies of auditory evoked potentials were found in small studies (n = 29, 49) of children and adults with higher blood Pb levels (i.e., range 6-84 µg/dL) than most of the current U.S. population of children ([Holdstein et al., 1986](#); [Robinson et al., 1985](#)).

Recent cross-sectional studies aimed to identify the locus in the auditory system affected by Pb exposure in the examination of a population of children (n = 53, 117, ages 2-18 years) living in Pb glazing communities in Ecuador with higher blood Pb levels than those relevant to this ISA (means 34 and 38 µg/dL) ([Buchanan et al., 2011](#); [Counter et al., 2011](#)). Concurrent blood Pb level was not correlated with the acoustic stapedius reflex ([Counter et al., 2011](#)) or distortion product otoacoustic emissions ([Buchanan et al., 2011](#)), indicating lack of effect on the auditory brainstem or inner ear, respectively. Other loci were not examined, and potential confounding was not considered.

Epidemiologic Studies of Auditory Function in Adults

Studies of auditory function reviewed in the 2006 Pb AQCD consistently indicated associations between blood Pb levels and prolonged latencies in auditory evoked brainstem potentials in occupationally-exposed adults ([U.S. EPA, 2006b](#)). A few recent studies found increases in hearing thresholds in Pb-exposed workers. A recent analysis of the NAS provided evidence in nonoccupationally-exposed men for associations of tibia Pb levels with hearing loss.

Among 448 men in the NAS, higher tibia Pb level (mean: 22.5 µg/g) at mean age 64.9 years, measured up to 20 years after initial hearing testing, was associated with a faster rate of increase in hearing threshold for frequencies of 1, 2, and 8 kHz and a pure tone average. Men were free of hearing loss at baseline and had hearing tested repeatedly (median 5 times per subject) over a median of 23 years ([Park et al., 2010](#)). Blood Pb was not examined. In cross-sectional analyses adjusted for age, race, education, body mass index, pack-years of cigarettes, diabetes, hypertension, occupational noise (based on a job-exposure estimate), and presence of a noise notch (indicative of noise-induced hearing loss), higher patella Pb level (mean 32.5 µg/g, measured within 5 years of hearing test) was associated with a higher hearing threshold for frequencies greater than 1 kHz. A 21 µg/g (interquartile range) increase in patella Pb level was associated with pure tone average hearing loss with an OR of 1.48 (95% CI: 1.14, 1.91) in adjusted analyses.

Similar, but slightly weaker associations were found for tibia bone Pb levels. In the NAS, bone Pb levels were measured after the initial hearing measurement but reverse causation is unlikely since bone Pb is an indicator of cumulative Pb exposure, and tibia Pb has a half-life on the order of decades ([Section 3.3](#)). Bone Pb levels increase with age, and although age was included as a model covariate, residual confounding by age is possible.

Recent cross-sectional studies added evidence for associations between higher concurrent blood Pb levels and higher hearing thresholds in adults with potential or actual occupational Pb exposures. A hospital-based case-control study examined workers from diverse occupations and examined potential confounding by other occupational exposures. Cases included workers referred for hearing testing (average hearing thresholds above 25 dB), and controls comprised workers with normal hearing thresholds who were having routine occupational health examinations ([Chuang et al., 2007](#)). Geometric mean blood Pb levels were 10.7 µg/dL for the 121 cases and 3.9 µg/dL for the 173 controls. In models that adjusted for age, smoking status, alcohol consumption, years of noise exposure, as well as Mn, arsenic (As), and selenium (Se) levels in blood, higher blood Pb levels were associated with higher hearing threshold (0.5-6 kHz). The potential selection bias arising from the nonrandom population sample may limit implications of these findings. Other studies found associations of higher concurrent blood Pb level with increased hearing thresholds or hearing loss in Pb-exposed workers (n = 183-259) but had limited or no consideration for potential confounding ([Hwang et al., 2009](#); [Forst et al., 1997](#)) and/or examined workers with a mean blood Pb level of 57 µg/dL ([Wu et al., 2000](#)).

Toxicological Studies of Auditory Function

The 2006 Pb AQCD ([U.S. EPA, 2006b](#)) described increases in hearing thresholds or latencies in brainstem auditory evoked potentials in nonhuman primates (n = 5-7) exposed to lifetime Pb from gestation or birth to ages 8-13 years (resulting in blood Pb levels 33-150 µg/dL at peak or during testing) ([Rice, 1997](#); [Lilienthal and Winneke, 1996](#)). Rice ([1997](#)) found that half of the pure tone detection thresholds were above the control range at certain frequencies. In Lilienthal and Winneke ([1996](#)), the Pb-related increased latency in auditory evoked potentials persisted after Pb exposure was terminated, and blood Pb levels had decreased to < 5 µg/dL. The observations in monkeys are consistent with the epidemiologic associations described above but were related to higher Pb exposures than those relevant to this ISA. In addition to indicating hearing loss, brainstem auditory evoked potentials can indicate impaired synaptic maturation and incomplete neuron axon myelination leading to impaired neuronal conduction ([Gozdzik-Zolnierkiewicz and Moszyński, 1969](#)). Thus, the findings from Rice ([1997](#)) and those described in the preceding sections for children may indicate that Pb

exposure impairs auditory nerve conduction. Studies in animals with blood Pb levels >300 µg/dL found that the cochlear nerve was especially sensitive to Pb exposure ([Gozdzik-Zolnierkiewicz and Moszyński, 1969](#)).

A recent study found similar effects in monkeys with similar blood Pb levels as Lilienthal and Winneke ([1996](#)), but with only early-life Pb exposure. Laughlin et al. ([2009](#)) studied rhesus monkeys exposed to Pb acetate prenatally to birth or postnatally from birth through weaning at age 5.5 months (maternal drinking water, 3 months prior to mating until weaning, resulting in bone Pb levels at age 11 years of 7 and 13 µg/g for prenatal and postnatal groups, respectively, and blood Pb levels averaged over Pb exposure of 35 and 46 µg/dL, respectively). Auditory threshold testing and threshold task testing was conducted at 13 years of age after blood Pb levels had returned to those found in controls. At birth, animals were cross fostered, creating a control group, a prenatal Pb group, and a postnatal Pb group; however, Pb-exposed animals were analyzed as a single group. Pb exposure induced small, statistically nonsignificant elevations in auditory thresholds in animals. Behavioral testing during the auditory threshold task indicated less engagement and responsiveness in Pb-exposed animals. This study has multiple limitations that could have contributed to the lack of statistically significant aberrations, including limited power with the examination of 5 animals per group, the inability of some of the monkeys to engage or focus on the task at hand which resulted in fewer available measurements, differences between the sexes in attention, and mixing of the postnatal Pb and prenatal Pb-exposed animals into one group.

Summary of Auditory Function

Children

Several studies indicated that higher blood Pb levels are associated with decrements in auditory function in children, as evidenced by increases in hearing thresholds. Results from the prospective Cincinnati cohort study (n = 215) provide key evidence for associations of prenatal maternal, neonatal (age 10 day), yearly age 1 to 4 year, and lifetime average blood Pb levels with increased hearing thresholds at age 57 months ([Dietrich et al., 1992](#)), and large (n = 3,000-4,000) cross-sectional analyses of children participating in NHANES and HHANES provide supporting evidence for concurrent blood Pb levels ([Schwartz and Otto, 1991, 1987](#)). The examination of multiple exposures and outcomes in NHANES and HHANES reduces the likelihood of participation conditional specifically on Pb exposure and hearing function. In the Cincinnati cohort, mean blood Pb levels were 4.8 µg/dL for neonatal and 17.4 µg/dL for lifetime average. In HHANES, the median concurrent blood Pb level was ~8 µg/dL. Across studies, associations were found with adjustment for factors such as age, sex, ethnicity, family

income, concurrent or past colds, antibiotic use, degree of urbanization, and Apgar score. Potential confounding by parental education, nutritional factors, environmental noise, and maternal smoking also was considered.

Mechanistic evidence was provided by observations of associations between blood Pb level with lower auditory evoked potentials in children, particularly associations found in the prospective analysis of children ages 5-7 years in Mexico City with prenatal maternal, age 1 year, and age 4 year blood Pb levels ([Rothenberg et al., 2000](#)). Pb-related increases in hearing thresholds and latencies in brainstem auditory evoked potentials also were found in nonhuman primates with lifetime Pb exposure beginning in gestation or birth ([Rice, 1997](#); [Lilienthal and Winneke, 1996](#)), although auditory assessments were made in adult animals ages 8-13 years. Shorter-duration Pb exposure from gestation to birth or from birth to age 5.5 months was found to induce small, statistically nonsignificant elevations in auditory thresholds and decreased responsiveness in behavioral testing performed with the threshold task ([Laughlin et al., 2009](#)). In animals, auditory effects were found with higher blood Pb levels (i.e., 33-150 µg/dL at peak or testing) than those relevant to this ISA; thus, it is difficult to ascertain biological plausibility for observations in children.

Adults

In adults, increased hearing thresholds and hearing loss were associated with bone Pb levels in 448 NAS men who were unlikely to have had occupational Pb exposures ([Park et al., 2010](#)) and with concurrent blood Pb levels in adults with potential occupational Pb exposure. In the NAS, the examination of multiple exposures and outcomes reduces the likelihood of participation conditional specifically on Pb exposure and auditory function. Among NAS men, higher tibia Pb levels were associated with a faster rate of increase in hearing thresholds over a 23 year follow-up with adjustment for age, race, education, body mass index, pack-years of cigarettes, diabetes, hypertension, occupational noise, and presence of a noise notch. Tibia Pb levels were measured up to 20 years after initial hearing testing, and while there is uncertainty regarding the temporal sequence between Pb exposure and changes in hearing thresholds, Pb in tibia has a half-life on the order of decades. Temporality also is difficult to establish in the cross-sectional occupational studies. A hospital-based case-control study found an association between higher concurrent blood Pb levels and higher hearing thresholds among workers with relevant blood Pb levels (means 10.7 and 3.9 µg/dL in workers with and without hearing problems, respectively) ([Chuang et al., 2007](#)). Among other factors, results were adjusted for other metal exposures. Similar auditory effects were found in adult animals ages 8-13 years with lifetime Pb exposures but with higher blood Pb levels (i.e., 33-150 µg/dL) than those relevant to this ISA.

4.3.6.2 Visual Function

The sections that follow review studies examining relationships of Pb exposure with visual function as related to effects on the central nervous system. Studies examining other effects on the visual system such as cataracts are described in [Section 4.9.5](#).

Epidemiologic Studies of Visual Function

The little available epidemiologic evidence does not clearly describe an effect of relevant Pb exposures with effects on the retina in humans. Among children (n = 100-113, ages 7-10 years) in Mexico City examined prospectively, Rothenberg et al. ([2002b](#)) reported an association between maternal first trimester blood Pb levels 10.5-32.5 µg/dL and supernormal electroretinograms (ERGs), the impact of which on visual impairment is not clear. In a case-control study of macular degeneration (condition due to retinal damage) in adults, Pb concentrations were elevated in retinal tissue of 36 cases (50 eye donors at time of autopsy; median [IQR]: 12.0 [8-18] ng/g Pb) versus 25 controls (72 eyes; median [IQR]: 8.0 [0-11] ng/g Pb) ([Erie et al., 2009](#)). The implications of results in children and adults are limited by the lack of rigorous statistical analysis and consideration for potential confounding. The macular degeneration results are limited further by the inability to establish directionality of effects and uncertainty in representativeness of the control population.

Toxicological Studies of Visual Function

As described in the 1986 and 2006 Pb AQCDs ([U.S. EPA, 2006b, 1986b](#)), a majority of the evidence for the effects of Pb on the visual system has been provided by toxicological studies. Pb exposure of animals during perinatal development and adulthood has been found to reduce visual acuity and produce changes describing potential mechanisms of action such as alterations in the retina ([Fox et al., 1997](#); [Fox and Sillman, 1979](#)), CNS visual processing areas ([Costa and Fox, 1983](#)), and subcortical neurons involved in vision ([Cline et al., 1996](#)). For example, environmentally-relevant doses of Pb (10^{-3} µM) in tadpoles inhibited the growth of neurons in the subcortical retinotectal pathway, the main efferent from the retina ([Cline et al., 1996](#)). Pb-related aberrations in electrical responses in retinal cells, as measured by ERGs, have been found in rodents and nonhuman primates. Recent research on ERGs expands upon the extant evidence by examining animals with lower Pb exposures or blood Pb levels.

Work in nonhuman primates with Pb exposure during development or over a lifetime (peak blood Pb levels 50-115 µg/dL) showed dysfunction in temporal visual function (responses to different frequencies of light flicker) under high luminance but no change

in spatial function ([Rice, 1998](#)). A recent study found no effects of infancy Pb exposure (PND8-age 26 weeks via commercial milk formula, producing blood Pb levels of 35-40 µg/dL) on spatial acuity of rhesus monkeys as assessed with the modified Teller preferential looking paradigm ([Laughlin et al., 2008](#)). In monkeys, effects on vision were tested with higher Pb exposures than those relevant to this ISA. Low-level developmental Pb exposure was found to result in sensorimotor deficits in adult zebrafish ([Rice et al., 2011](#)). Fish that were exposed as embryos (2 to 24 hours post-fertilization) to water containing 0.03 µM PbCl₂ had impaired response to visual stimulation (a rotating bar) under low light conditions. These zebrafish also failed to respond normally to mechanosensory stimulation (0.01 and 0.03 µM PbCl₂), showing a significantly impaired startle response.

Animal toxicological evidence also shows that the lifestage of exposure and the dose of Pb contribute to the complex and variable effects of Pb on ERGs (summarized in [Table 4-13](#)). The biological relevance of these variable findings is uncertain. Female rats exposed postnatally to 200 or 2,000 ppm Pb acetate exposure via dam drinking water from birth through lactation, resulting in blood Pb levels of 19 and 59 µg/dL at weaning, respectively, had subnormal scotopic ERGs (decreased A- and B-wave amplitudes) with decreased sensitivity and temporal resolution when assessed at 90 days of age ([Fox et al., 1991](#)). Similar results were obtained in multiple studies conducted in in vitro models ([Otto and Fox, 1993](#); [Fox and Farber, 1988](#); [Fox and Chu, 1988](#)). Monkeys exposed to relatively high levels of Pb continuously from the prenatal period to age 7 years (350 or 600 ppm Pb acetate, resulting in blood Pb levels of 40 and 50 µg/dL, respectively) had persistently increased maximal ERG amplitude (B-wave only, supernormality) and increased mean ERG latency when assessed 2 years after Pb exposure was terminated when blood Pb levels were <10 µg/dL ([Lilienthal et al., 1988](#)).

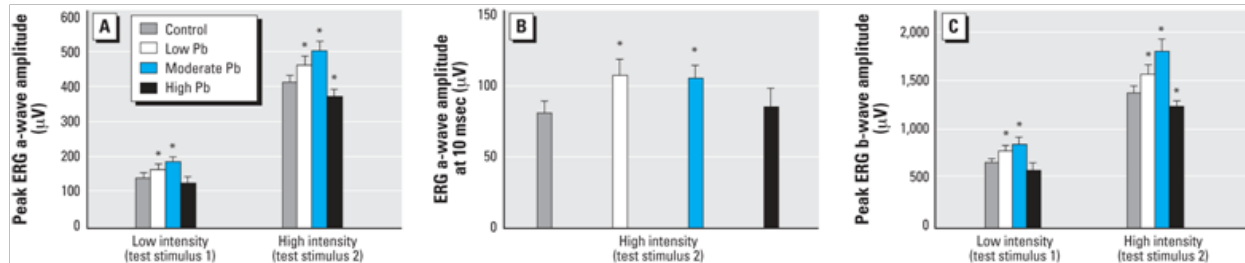
Table 4-13 Summary of Pb-related effects observed on the visual system.

Study, Species, Sex	Pb Exposure Protocol and Dose	Maximal Blood Pb Level (µg/dL)	ERG Effect	Progenitor Cell Proliferation	Retinal Cell Apoptosis	Retinal Dopamine Levels	Retinal Cell Layer Thickness
Fox et al. (2008) Long-Evans Rat, F	DW, Prenatal-PND10						
	Low, 27 ppm	12	Supernormal	Yes	Not affected	Dose-dependent ↓	↑
	Moderate, 55 ppm	24	Supernormal	Yes	Not affected	Dose-dependent ↓	↑
	High, 109 ppm	46	Subnormal	No	Yes	Dose-dependent ↓	↓
Lilienthal et al. (1988) Rhesus Monkey, M & F	DW, Prenatal-lifetime						
	350 ppm	~50	Supernormal	—	—	—	—
	600 ppm	~115	Supernormal	—	—	—	—
Fox et al. (1997) Long-Evans Rat, F	DW, PND1-21						
	200 ppm	19	Subnormal	—	Yes	—	↓
	2,000 ppm	59	Subnormal	—	Yes	—	↓
Rothenberg et al. (2002b) Human children, M & F	Prenatal maternal 1st trimester blood	10.5-32.5	Supernormal	—	—	—	—

F = females, M = males, DW = Drinking water exposure, - denotes not examined in the study.

Pb exposure beginning in the gestational period (Pb acetate in drinking water from 2 weeks before mating to PND10) also induced supernormal ERGs in adult Long-Evans rats, with low (27 ppm) and moderate (55 ppm) doses that produced blood Pb levels 10-12 µg/dL and 21-24 µg/dL (Fox et al., 2008) (Figure 4-11 and Table 4-13). This exposure window represents the developmental period for the retina of the rat and is analogous to gestational human retinal development. Subnormal ERGs were induced by the high 109 ppm dose (Figure 4-11), which produced blood Pb levels 40-46 µg/dL. Results of this rodent study demonstrated persistent supernormal scotopic rod photoreceptor-mediated ERGs in animals with blood Pb levels relevant to this ISA. These findings were consistent with the association observed between supernormal ERGs and prenatal maternal blood Pb levels 10.5-32.5 µg/dL in male and female children (Rothenberg et al., 2002b). The functional relevance of findings is uncertain as

supernormal scotopic ERGs may be recorded without other overt ophthalmologic changes and are rarely seen in the clinical setting ([Terziivanov et al., 1982](#)).



Note: *p < 0.05. Low Pb = 27 ppm Pb acetate, blood Pb level 10-12 µg/dL, Moderate Pb = 55 ppm, blood Pb level 21-24 µg/dL, High Pb = 109 ppm, blood Pb level 40-46 µg/dL. Relative to controls (gray bars), low (white bars) or moderate (blue bars) Pb exposure from gestation through postnatal day (PND)10 induced supernormal ERGs whereas high Pb exposure (black bars) induced subnormal ERGs.

Source: Fox et al. ([2008](#))

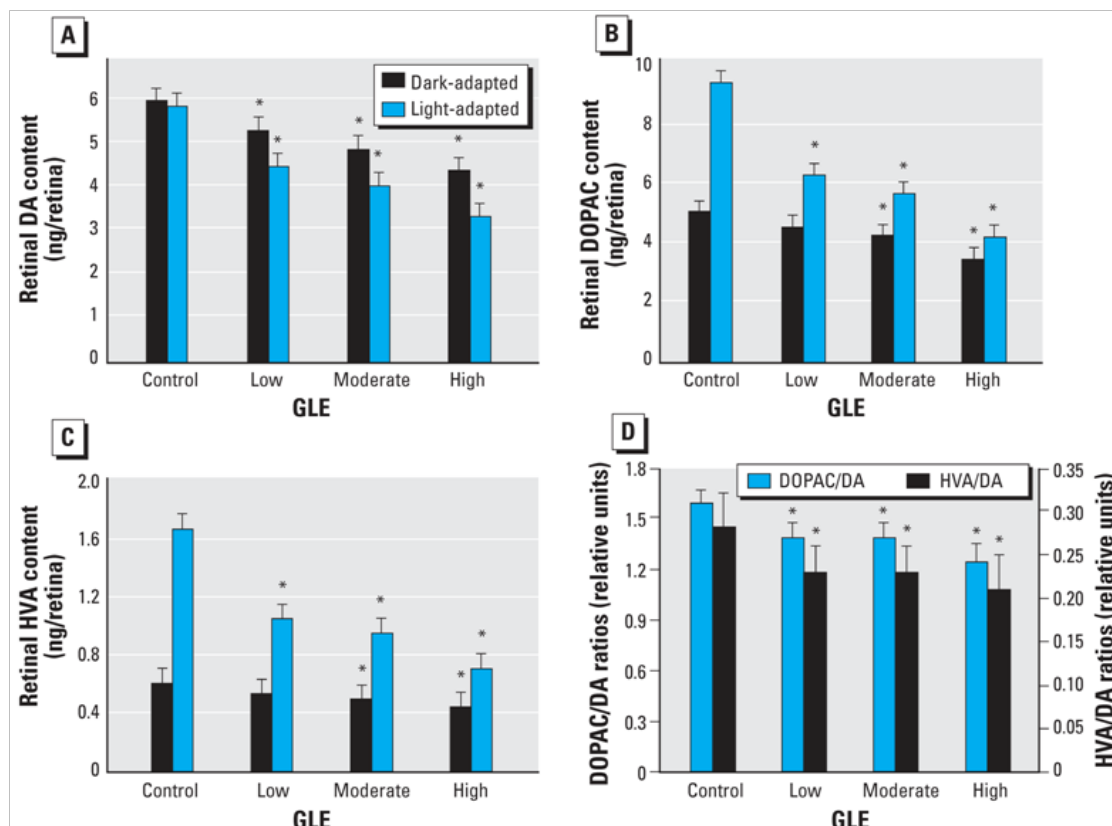
Figure 4-11 Retinal a-wave and b-wave electroretinogram (ERG) amplitude in adult rats after prenatal plus early postnatal Pb exposure.

Animal studies indicate that the dose of Pb and the exposure lifestage not only differentially affect functional tests, i.e., ERG, but also differentially affect retinal cell numbers and morphology. Concomitant with Pb-induced supernormal ERGs, Fox et al. ([2008](#)) found that 27 and 55 ppm gestational plus early postnatal Pb exposure increased neurogenesis of rod photoreceptors and rod bipolar cells without affecting apoptosis of Müller glial cells and increased the number of rods in central and peripheral retina ([Table 4-13](#)). Concomitant with subnormal ERGs, higher-level gestational plus early postnatal Pb exposure (109 ppm, blood Pb level 40-46 µg/dL) decreased the number of rods in the central and peripheral retina ([Fox et al., 2008](#)). Early postnatal (PND1-PND21) Pb exposure (200 or 2,000 ppm, producing blood Pb levels 19 and 59 µg/dL) induced scotopic ERG subnormality in adult rats, decreased the number of rods in the central and peripheral retina, and decreased the retinal Zn concentration ([Fox et al., 1997](#)) ([Table 4-13](#)). Similar observations were made in separate work in mice. Low and moderate doses of Pb from gestation to PND10 (27 or 55 ppm Pb acetate in dam drinking water, producing blood Pb levels of 12 and 25 µg/dL, respectively) induced greater and prolonged rod bipolar cell neurogenesis and greater thickness and cell number of the outer and inner neuroblastic layers of the retina ([Giddabasappa et al., 2011](#)). As in rats, with a higher dose of Pb (109 ppm Pb acetate, resulting in blood Pb levels of 56 µg/dL), there was no increased rod neurogenesis in mice. Nitric oxide has been shown to regulate retinal progenitor cell proliferation in chick embryos ([Magalhaes et al., 2006](#)). Thus,

these authors postulated that impaired NO production may contribute to aberrant retinal cell proliferation ([Giddabasappa et al., 2011](#)).

Mechanistic understanding of the effect of Pb on the visual system includes the capability of Pb to displace divalent cations, inhibit physiological enzymes, alter cell proliferation and apoptosis, perturb normal neuroanatomical formation (cytoarchitecture in the brain), and affect neurotransmitters. The effects of Pb on the retina were shown to be mediated by inhibition of cGMP phosphodiesterase (PDE) by Pb ([Srivastava et al., 1995](#); [Fox and Farber, 1988](#)). Without regard to Pb exposure, pharmacological inhibition of cGMP PDE has been linked with visual problems including alterations in scotopic ERGs ([Laties and Zrenner, 2002](#)). Postnatal Pb exposure of animals (peak blood Pb levels: 19, 59 µg/dL) or in vitro Pb exposure of rods isolated from these animals elevated cGMP which contributed to elevated rod calcium concentration ([Fox and Katz, 1992](#)) and subsequently induced apoptotic cell death in a concentration-dependent manner.

Pb has been shown to affect a plethora of neurotransmitters in the brain, and it has recently been shown to affect neurotransmitters in the retina. With the aforementioned gestational to PND10 exposure, Pb induced a concentration-dependent decrease in adult rat retinal synthesis of dopamine, which has functions in retinal growth and development and signal transduction in rods and cones ([Fox et al., 2008](#)) ([Figure 4-12](#)). As discussed in the 2006 Pb AQCD ([U.S. EPA, 2006b](#)), other mode of action support for the effects of Pb on the visual system is provided by observations of Pb-induced decreased Na⁺/K⁺ATPase activity which have been reported in vitro and in vivo. Also, structural changes from chronic Pb exposure (birth to age 6 years) of monkeys included cytoarchitectural changes in visual projection areas of the brain. Maximum blood Pb levels in the low and high dose groups reached 20 µg/dL and 220 µg/dL, respectively ([Reuhl et al., 1989](#)).



Note: *p < 0.05. GLE = Gestational Pb exposure to postnatal day 10. Low Pb = 27 ppm Pb acetate, blood Pb levels 10-12 µg/dL, Moderate Pb = 52 ppm, blood Pb levels 21-24 µg/dL, High Pb = 109 ppm, blood Pb levels 40-46 µg/dL. A. DA = dopamine, B. DOPAC = dopamine metabolite, C. HVA = dopamine metabolite, D. DOPAC/DA and HVA/DA = ratio of dopamine metabolite to dopamine. Pb exposure decreased dopamine, DOPAC, and HVA in a concentration-dependent manner in light-adapted animals (blue bars). In dark adapted animals (black bars), Pb exposure decreased dopamine, DOPAC, and HVA but not always in concentration-dependent manner. Pb exposure decreased DOPAC/DA and HVA/DA.

Source: Fox et al. (2008)

Figure 4-12 Retinal dopamine metabolism in adult control and gestationally Pb-exposed (GLE) rats.

Summary of Visual Function

Increasing maternal first trimester blood Pb levels (10.5-32 µg/dL) were associated with supernormal ERGs in children in Mexico City at ages 5-7 years (Rothenberg et al., 2002b). The animal evidence showed ERGs in different directions depending on lifestage of Pb exposure and blood Pb level. Supernormal ERGs were found in adult rats with prenatal plus early postnatal (PND10) Pb exposure that produced blood Pb levels of 12 and 24 µg/dL (Fox et al., 2008). The implication of supernormal ERGs on visual impairment is not clear, and the biological relevance of the nonlinear concentration-response is not clear. In these animals, Pb exposure also increased rod cell neurogenesis

and decreased dopamine. Toxicological studies also found other retinal effects including alterations in morphology and cell architecture, signaling, enzyme inhibition, neurotransmitter levels, neuroanatomical development, cell proliferation, and retinal cell apoptosis. Adult monkeys were found to have supernormal ERGs and decreased temporal visual function with developmental or lifetime Pb exposure ([Rice, 1998](#)), but infant monkeys had no change in visual acuity with infancy Pb exposure ([Laughlin et al., 2008](#)). In each case, Pb exposures were higher (blood Pb levels ≥ 35 $\mu\text{g/dL}$) than those relevant to this ISA. Pb-associated visual system effects in human adults are not well characterized and are limited to a case-control study finding higher Pb concentrations in retinal tissue from macular degeneration cases ([Erie et al., 2009](#)) that did not perform rigorous statistical analysis or examine potential confounding factors.

4.3.7 Motor Function

Some studies in children have assessed fine motor function, i.e., response speed, dexterity, as part of a battery of neurodevelopmental testing, and most have found associations with blood Pb level. Fewer studies have examined gross motor function, i.e., postural balance, action tremor, agility, but also have found associations with blood Pb level in children. Poorer motor function also was found in Pb-exposed workers.

Key evidence from prospective analyses of the Cincinnati and Yugoslavia cohorts demonstrated associations of blood Pb levels with poorer motor function with either adjustment for or consideration for several potential confounding factors related to SES, parental caregiving quality and education, smoking exposure, birth outcomes, sex, and child health. In the Cincinnati cohort, higher earlier childhood blood Pb levels (age 0-5 year average [median: 11.7 $\mu\text{g/dL}$] or age 78 month) were associated with poorer fine (n = 195) ([Ris et al., 2004](#)) and gross motor function (n = 91) ([Bhattacharya et al., 2006](#)) assessed in adolescence (ages 12, 15-17 years). In this cohort recruited from birth, follow-up participation was high and not conditional on blood Pb levels. The fine motor function results were adjusted for maternal IQ, SES, HOME score, sex, and adolescent marijuana consumption. Collectively, these findings suggest the persistence of effects of earlier childhood Pb exposure to later childhood; however, later childhood or concurrent blood Pb levels were not examined. Assessments in Cincinnati cohort children at age 6 years indicated associations of concurrent (mean: 10.1 $\mu\text{g/dL}$), lifetime average (mean: 12.3 $\mu\text{g/dL}$), and neonatal (mean: 4.8 $\mu\text{g/dL}$) but not prenatal maternal (mean: 8.4 $\mu\text{g/dL}$) blood Pb levels with poorer upper limb dexterity, fine motor composite score (n = 245) ([Dietrich et al., 1993a](#)), and poorer postural balance (n = 162) ([Bhattacharya et al., 1995](#)). These results were adjusted for HOME score and race. Additional covariates included maternal IQ, SES, and sex for fine motor functions ([Dietrich et al., 1993a](#)) and height,

BMI, birth weight, bilateral ear infection, nutritional factors, and foot area for postural balance ([Bhattacharya et al., 1995](#)). Blood Pb levels were associated with fine and gross motor function in unadjusted and adjusted analyses, increasing confidence that confounding by the examined covariates did not unduly bias the observed associations. Prospective analysis of the Yugoslavian cohort indicated an association of lifetime average blood Pb level (exact levels not reported) with decrements in fine but not gross motor function at age 4.5 years in 283 children ([Wasserman et al., 2000a](#)). Although only 50% of the cohort was examined and participation was greater among children with lower SES and HOME score, participation was not conditional on higher blood Pb levels.

Evidence from cross-sectional studies for associations between motor function and concurrent blood Pb level was mixed, with studies examining mean concurrent blood Pb levels 2-3 µg/dL not finding decrements in motor function. Surkan et al. ([2007](#)) found that higher concurrent blood Pb level was associated with better fine motor function as indicated by faster finger tapping speed among 534 children (ages 6-10 years) in New England. Results were adjusted for several potential confounding factors such as age, sex, caregiver IQ, SES, and race. Min et al. ([2007](#)) found weak, imprecise associations in 61 similarly aged children (7-16 years) in Korea. Concurrent blood Pb level (mean: 5.0 µg/dL) was associated with greater sway oscillation, action tremor, and alternating arm movements in 110 Inuit children (ages 4-6 years) in Quebec, Canada ([Despres et al., 2005](#)) with consideration for potential confounding by factors such as HOME score, maternal education and several nutrient levels. The population of Inuit children was selected from subsistence fishing communities, who also have higher exposure to Hg and PCBs. Several indices of fine and gross motor function were associated with blood Pb level, with adjustment for these other exposures. Poorer fine motor function also was associated with higher concurrent blood Pb level in 814 children ages 3-7 years in India with mean concurrent blood Pb level 11.4 µg/dL with adjustment for similar factors as the aforementioned studies ([Palaniappan et al., 2011](#)).

An association of Pb exposure with poorer motor function in adults was found in Pb-exposed workers ([Iwata et al., 2005](#)), although implications are limited by the cross-sectional design, high concurrent blood Pb levels (mean: 40 µg/dL), and lack of consideration for potential confounding by other occupational exposures. Among 121 Pb smelter workers in Japan, higher blood Pb level was associated with greater sagittal sway with eyes open ($p < 0.05$) and eyes closed ($p < 0.01$) and transversal sway with eyes closed ($p < 0.05$) with adjustment for age, height, smoking status, and drinking status. A mean benchmark dose level ([Budtz-Jorgensen et al., 2001](#); [NRC, 2000](#)) of 14.3 µg/dL was calculated for postural sway from a linear model. A supralinear concentration-response function fit the data slightly better than a linear function did.

Pb exposure has shown mixed effects on endurance, balance, and coordination in animals as measured by rotarod performance, treadmill testing, and visual discrimination task. Lower concentration gestational plus early postnatal (to PND10) Pb exposure (27 ppm, producing peak blood Pb level ≤ 10 $\mu\text{g/dL}$ at PND0-PND10) resulted in significantly poorer rotarod performance (i.e., falling off more quickly) than did higher exposure (109 ppm, blood Pb level: 33-42 $\mu\text{g/dL}$) in male (but not female) adult mice, indicative of a nonlinear concentration-response relationship ([Leasure et al., 2008](#)). Other rotarod experiments examining various speeds of rotarod rotation, higher than relevant Pb exposures (blood Pb levels >59 $\mu\text{g/dL}$), and/or routes of exposure with uncertain relevance to humans, yielded mixed results ([Kishi et al., 1983](#); [Grant et al., 1980](#); [Overmann, 1977](#)). An effect on motor function was indicated by increased alcove latency of rats performing a visual discrimination task but with Pb exposure (PND1-30) blood Pb levels of 31 $\mu\text{g/dL}$ ([Stangle et al., 2007](#)). Herring gull chicks injected with a single i.p. bolus dose of Pb (100 mg/kg Pb acetate, selected to represent exposure in the wild) had slower development of motor skills versus control birds, as assessed by the treadmill test ([Burger and Gochfeld, 2005](#)).

In summary, epidemiologic evidence demonstrates associations of higher blood Pb levels with poorer fine and gross motor function in children ages 3-17 years. Little evidence is available in adults. Prospective analyses of the Cincinnati and Yugoslavia cohorts (n = 91-283) that considered several potential confounding factors such as SES and child health found associations with earlier childhood blood Pb levels (i.e., age 78 month, 0-5 year average) in adolescents ([Bhattacharya et al., 2006](#); [Ris et al., 2004](#)) and neonatal, lifetime average, and concurrent blood Pb levels in children ages 4-6 years ([Wasserman et al., 2000b](#); [Bhattacharya et al., 1995](#); [Dietrich et al., 1993a](#)). In the Cincinnati cohort, neonatal blood Pb levels were lower than concurrent or lifetime average blood Pb levels at age 6 years (means 4.8, 10.1, 12 $\mu\text{g/dL}$, respectively). In cross-sectional studies that examined similar potential confounding factors, results were inconsistent in populations with mean blood Pb levels 2-5 $\mu\text{g/dL}$ ([Min et al., 2007](#); [Surkan et al., 2007](#); [Despres et al., 2005](#)). While Pb exposure had mixed effects on motor function in animals, mice with relevant blood Pb levels (peak ≤ 10 $\mu\text{g/dL}$) after gestational-early postnatal Pb exposure showed poorer balance ([Leasure et al., 2008](#)).

4.3.8 Seizures in Animals

Previous studies did not consistently show that Pb exposure induced seizures in animals. Pb-induced seizures were found in male Wistar rats exposed to Pb acetate postnatally (250-1,000 ppm in drinking water PND60-PND90, resulting in blood Pb levels of 20-43 $\mu\text{g/dL}$), as indicated by a decrease in the elapsed time required to develop the first

myoclonic jerk and tonic-clonic seizure ([Arrieta et al., 2005](#)). Also, the dose of the seizure-inducing agent pentylenetetrazol (PTZ) required to induce seizures significantly decreased in all Pb dose groups. Other studies showed no effect of Pb exposure on seizures ([Schwark et al., 1985](#); [Alfano and Petit, 1981](#)). In a study of early postnatal Pb acetate exposure (2,000 ppm in drinking water PND1-PND25), Pb had variable effects on induction of seizures in Sprague Dawley rats examined at PND25 or PND50, depending on the convulsant-inducing agent administered ([Chen and Chan, 2002](#)). These variable effects were hypothesized to be due to the selective effects on inhibitory and excitatory neurotransmission by age and blood Pb level, which were 47 µg/dL and 11 µg/dL at PND25 and PND50, respectively.

Recent investigation expanded on the work by Arrieta et al. ([2005](#)) by showing that Pb exposure may induce seizure activity in another rodent species, BALB/c mice. Adult (ages 2-3 months) male BALB/c mice were exposed to Pb acetate for 30 days via drinking water (range of blood Pb levels 50-400 ppm Pb groups: 6.4-18 µg/dL) ([Mesdaghinia et al., 2010](#)). Except for 50 ppm Pb exposure, all other Pb concentrations significantly reduced the thresholds of face and forelimb clonus, myoclonic twitch, running and bouncing clonus, and tonic hindlimb extension. High-level Pb administered by bolus injection (200 mg/kg Pb acetate or 50 mg/kg Pb nitrate, single injection, 2 days, blood Pb levels >90 µg/dL) also induced epileptic form activity or seizures in adult male Wistar rats ([Krishnamoorthy et al., 1993](#)).

4.3.9 Neurodegenerative Diseases

4.3.9.1 Alzheimer's Disease

Lower scores on the MMSE, which is widely used as a screening tool for dementia, have been associated with higher bone Pb level in NAS men ([Wang et al., 2007a](#); [Weisskopf et al., 2004](#); [Wright et al., 2003](#)) but inconsistently with blood Pb level in adults ([Weuve et al., 2006](#); [Nordberg et al., 2000](#)). Direct evidence regarding the effects of Pb exposure on Alzheimer's disease is limited to studies reviewed in the 2006 Pb AQCD ([U.S. EPA, 2006b](#)), which did not find higher occupational exposure to Pb ([Graves et al., 1991](#)) or Pb level in the brains ([Haraguchi et al., 2001](#)) in Alzheimer's disease cases than unaffected controls. Overall, the latter studies have sufficient limitations (e.g., case-control design that may be subject to reverse causation, limited consideration for potential confounding) such that evidence is inconclusive regarding the effect of Pb exposure on Alzheimer's disease.

Despite inconclusive epidemiologic evidence, toxicological evidence indicates that Pb exposure in early life promotes Alzheimer's disease-like pathologies in the brains of aged adult animals. Alzheimer's disease is characterized by amyloid-beta peptide (Ab) plaques, hyper-phosphorylation of the tau protein, neuronal death and synaptic loss. In the last decade, the developmental origins of adult health and disease paradigm and the similar Barker hypothesis have indicated that early life exposures can produce aberrant effects in adults. Bolin et al. ([2006](#)) connected oxidative DNA damage in adult rats with indicators of plaque formation after lactational Pb exposure that produced blood Pb levels of 46 µg/dL. Wu and colleagues ([2008a](#)) made similar observations with infantile Pb exposure of monkeys. These results suggest the need to directly examine the long-term effects of developmental exposure to toxicants rather than relying on adult exposure alone to predict potential health risks in adults ([Dietert and Piepenbrink, 2006](#)).

The fetal basis of amyloidogenesis has been examined extensively by the Zawia laboratory in both rodents and nonhuman primates. Mechanistically, Ab plaques originate from the cleavage of the amyloid precursor protein (APP). In male rats exposed to Pb as infants (200 ppm Pb acetate PND1-PND20 in dam drinking water, resulting in pup PND20 blood Pb level of 46 µg/dL and cortex 0.41 µg/g wet weight of tissue) or as adults, infancy Pb exposure induced APP gene expression in the aged animal brains ([Basha et al., 2005](#)). A bimodal response was observed, with a significant increase in APP expression above that in control animals first manifesting in infancy and again in old age (20 months). A concomitant bimodal response was observed in specificity protein 1 (Sp1), a transcription factor involved in gene expression in the early development of an organism and known to be related to APP expression. Ab was also significantly elevated in the aged animals developmentally exposed to Pb. Adult-only (age 18-20 months) exposure to Pb did not alter APP or Sp1 expression or Ab production.

Consistent with findings in rodents, Wu et al. ([2008a](#)) found that Pb exposure in infancy (PND1-PND400, 1.5 mg/kg per day in infant formula) resulted in significantly higher gene expression of APP and Sp1 and significantly higher protein expression of APP and Ab in aged female monkey cortex tissue (23 year-old *Macaca fascicularis*) from a cohort of animals established in the 1980s by Rice ([1992a](#), [1990](#)). At PND400, the monkeys had blood Pb levels of 19-26 µg/dL. In old age when amyloid plaques had manifested, blood Pb levels and brain cortex Pb levels had returned to control levels. Together, the rodent and nonhuman primate evidence concurs, and indicates that developmental Pb exposure and not adult-only exposure induces elevations in neuronal Alzheimer's Disease-related plaque proteins in aged animals.

Mechanistic understanding of Ab production and elimination after Pb exposure was examined in human SH-SY5Y neuroblastoma cells exposed to Pb concentrations of 0, 5,

10, 20, and 50 μM for 48 hours. Pb was found to affect two separate pathways to increase Ab. Pb exposure induced both the overexpression of APP and repression of neprilysin, a rate-limiting enzyme involved in Ab metabolism or removal ([Huang et al., 2011a](#)). Further mechanistic understanding of how Ab peptide formation is affected by Pb exposure was provided by Behl et al. ([2009](#)). The choroid plexus is capable of removing beta-amyloid peptides from the brain extracellular matrix. Pb was shown to impair this function, possibly by inhibiting the metalloendopeptidase, insulin-degrading enzyme, which metabolizes Ab ([Behl et al., 2009](#)). In another study, lactational Pb exposure of Long-Evans hooded rat pups induced perturbations in DNA binding of SP1 via its Zn finger protein motif ([Basha et al., 2003](#)). This effect of Pb was ameliorated by exogenous Zn supplementation.

An additional study with gestational plus lactational Pb exposure (1,000-10,000 ppm, dam drinking water, resultant offspring blood Pb levels: 40-100 $\mu\text{g/dL}$) showed that the rodent hippocampus as early as PND21 contained neurofibrillary changes, commonly used a marker for Alzheimer's disease. These changes manifested as hyperphosphorylated Tau, which comprises neurofibrillary tangles, and increased tau and beta amyloid hippocampal protein levels ([Li et al., 2010b](#)). Neurofibrillary changes were accompanied by poorer performance in tests of learning and memory.

In summary, recent studies showed that Pb exposure of rats and monkeys during infancy or during gestation/lactation induced significant increases in neuronal plaque-associated proteins such as Ab-peptide, activation of Ab-supporting transcription factors, and hyperphosphorylation of tau, all of which are pathologies found in humans with Alzheimer's disease. These pathologies were not found with adult-only Pb exposure of animals, further demonstrating that early life Pb is a critical window for Pb-induced Alzheimer's-like pathologies in animals. The few epidemiologic studies have not linked higher Pb exposure with Alzheimer's disease. These case-control studies lacked consideration for potential confounding. The animal evidence indicates that epidemiologic studies assessing concurrent brain Pb levels or occupational Pb exposure may not have examined the etiologically relevant exposure period. However, the observations that were made in experimental animals with high Pb exposure and blood Pb levels (40-100 $\mu\text{g/dL}$) may have uncertain relevance to this ISA. Further, animals were not behaviorally assessed for dementia.

4.3.9.2 Amyotrophic Lateral Sclerosis

The 2006 Pb AQCD ([U.S. EPA, 2006b](#)) reported mixed epidemiologic findings for an association between Pb and ALS based on case-control studies, several of which relied on

indirect methods of assessing Pb exposure. Case-control studies that measured blood Pb levels produced contrasting results. A study of 16 ALS cases (mean blood Pb level: 12.7 µg/dL) and 39 controls (mean blood Pb level: 10.8 µg/dL) found a small difference in the mean concurrent blood Pb level ([Vinceti et al., 1997](#)). A larger study of 109 cases and 256 controls that examined concurrent blood and bone Pb levels in a New England-area population found higher odds of ALS among subjects with concurrent blood Pb levels 3-14 µg/dL (e.g., OR: 14.3 [95% CI: 3.0, 69.3] for n = 55 with blood Pb levels 3-4 µg/dL compared with blood Pb levels <1-2 µg/dL) ([Kamel et al., 2002](#)). Odds of ALS were elevated in subjects in the highest two tertiles of patella and tibia Pb levels (≥ 10 µg/g patella Pb and ≥ 8 µg/g tibia Pb), but lacked precision. For example, compared with subjects with tibia Pb level <7 µg/g, the OR for tibia Pb levels 8-14 µg/g was 1.6 (95% CI: 0.5, 5.6). Results were adjusted for age, education, and hours/day inactive. Potential confounding by smoking was not considered. Also in this population, an estimate of cumulative Pb exposure based on occupational history was found to be associated with ALS ([Kamel et al., 2002](#)). The stronger findings for blood Pb level were surprising given that bone Pb level is a better biomarker of cumulative Pb exposure. One explanation for these findings is reverse causation. Blood was collected from people who already had ALS, and reduced physical activity among those with ALS could lead to more bone turnover and greater release of Pb from bones into circulation in ALS cases than controls.

Since the 2006 Pb AQCD, a few additional studies of ALS have been conducted with the same New England-area case-control group. Kamel et al. ([2005](#)) reported that the association between blood Pb level and ALS was not modified by the ALAD genotype, and Kamel et al. ([2008](#)) found that higher tibia and patella Pb levels were associated with longer survival time among 100 of the original 110 ALS cases with adjustment for age, sex, and ever smoking. Results were not altered by the additional adjustment for education, BMI, family history of ALS, or concurrent physical activity. Higher blood Pb levels were not associated with survival time. These paradoxical findings that point to a protective effect of Pb are not easily explainable but find coherence with results for Pb-induced increased survival time in an ALS mouse model (see below). On one hand, the cases with longer survival time may have higher bone Pb levels because they reflect a longer period of cumulative exposure. On the other hand, with longer survival time, there could be greater progression of disease and less mobility. Decreased mobility would tend to increase bone resorption, lower bone Pb levels, and increase blood Pb levels over time. The latter hypothesis is a less likely explanation for findings in this New England cohort because higher bone Pb levels were more strongly associated with longer survival time than was blood Pb level.

Another case-control study examined concurrent blood Pb levels and ALS among 184 cases (33 were either progressive muscular atrophy or primary lateral sclerosis, mean blood Pb level: 2.41 µg/dL) and 194 controls (mean blood Pb level: 1.76 µg/dL) ([Fang et al., 2010](#)). The cases were recruited from the National Registry of U.S. Veterans with ALS, and controls were recruited from among U.S. Veterans without ALS and frequency matched by age, gender, race, and past use of the Veterans Administration system for health care. A doubling of concurrent blood Pb level was associated with ALS with an OR of 1.9 (95% CI: 1.3, 2.7) with adjustment for age and a collagen protein as an indicator of bone formation. The association did not differ substantially by the degree of bone turnover but was slightly higher among ALAD 1-1 carriers. The association with blood Pb level was similar in analyses that excluded the progressive muscular atrophy and primary lateral sclerosis cases. The similar results by degree of bone turnover suggest that reverse causation is not likely explaining the association between blood Pb level and ALS. However, as in other ALS case-control studies, the directionality of effects is difficult to establish. This study did not have measures of bone Pb to assess the association with biomarkers of longer-term Pb exposure.

Although epidemiologic studies have provided inconsistent evidence for associations of Pb biomarker levels with ALS in adults, toxicological studies have found that Pb exposure affects neurophysiologic changes associated with ALS. For example, chronic postnatal Pb exposure from weaning onward (200 ppm Pb acetate in drinking water, resultant blood Pb level: 27 µg/dL) reduced astrocyte reactivity and induced increased survival time in the superoxide dismutase transgenic (SOD1 Tg) mouse, which has SOD mutations found in humans with familial ALS ([Barbeito et al., 2010](#)). In this model, Pb exposure did not significantly increase the onset of the ALS disease. These findings provide coherence with the association observed between bone Pb level and longer survival time in patients diagnosed with ALS ([Kamel et al., 2008](#)).

Research outside of the Pb field has suggested different mechanisms for ALS initiation versus ALS progression, i.e., motor neuron function versus astrocyte and microglia function ([Yamanaka et al., 2008](#); [Boillee et al., 2006](#)). Astrocyte vascular endothelial growth factor (VEGF) was examined for its involvement in the effects of Pb on increasing survival time in the ALS mouse model. Lower VEGF expression has been linked with risk of ALS in humans and ALS-like symptoms in animals. Baseline VEGF levels were elevated in astrocytes from the ventral spinal cord of untreated SOD1 Tg mice versus untreated nontransgenic mice. VEGF was not induced in the astrocytes of Pb-treated nontransgenic mice. In comparison, Pb-exposed SOD1 Tg mice, which had longer survival time, also had significant elevations in astrocyte VEGF ([Barbeito et al., 2010](#)). These findings for Pb-induced effects on astrocytes in a mouse model for ALS may provide a mechanistic explanation for Pb effects on increasing survival time in ALS.

Others reported that VEGF administration to the SOD1 Tg mice significantly reduced glial reactivity, a marker of neuroinflammation ([Zheng et al., 2007](#)). Using a cell-based co-culture system of neurons and astrocytes isolated from Pb-exposed SOD1 Tg mice, Barbeito et al. ([2010](#)) found that an up-regulation of VEGF production by astrocytes was protective against motor neuron death in the SOD1 Tg mouse cells. Thus, in vivo and in vitro results indicate that chronic Pb exposure resulted in increased survival time in an ALS mouse model and was correlated with higher spinal cord VEGF levels, which made astrocytes less cytotoxic to surrounding motor neurons ([Barbeito et al., 2010](#)).

In summary, there is inconsistent evidence of association between indicators of Pb exposure (history of occupational exposure, Pb biomarker levels) and ALS prevalence and survival time in humans. Other limitations include the potential for reverse causation and bias due to survival time in the case-control studies and the lack of objective assessment of occupational exposure. While several studies have considered potential confounding by age, education, and physical activity, few have considered smoking. Toxicological evidence also points to Pb exposure increasing survival in a mouse model of ALS and has suggested explanations including Pb-induced increases in VEGF expression and subsequent reduction in glial activity and protection of motor neurons against inflammation.

4.3.9.3 Parkinson's Disease

Previous Pb AQCDs reviewed a few studies, some ecological ([Rybicki et al., 1993](#); [Aquilonius and Hartvig, 1986](#)) and some case-control relying on questionnaire data or occupational history ([Gulson et al., 1999](#); [Gorell et al., 1997](#); [Tanner et al., 1989](#)) that indicated associations between exposure to heavy metals, particularly Pb, and risk of Parkinson's disease. The limited number of previous studies, weak study designs, and weak Pb exposure assessment did not permit firm conclusions. Recent studies maintain several of these limitations but have indicated associations with bone Pb levels.

A recent large case-control study (330 cases, 308 controls) examined a population in the Boston, MA area with virtually no occupational exposures to Pb ([Weisskopf et al., 2010](#)). Subjects in the highest quartile of tibia Pb level ($>16.0 \mu\text{g/g}$) had higher odds of Parkinson's disease compared to those in the lowest quartile ($\leq 3.1 \mu\text{g/g}$) (OR: 1.91 [95% CI: 1.01, 3.60]) with adjustment for age, race, pack-years smoking, education, and recruitment site. Cases and controls were recruited from several different sources including movement disorder clinics and the NAS, which could have introduced biased participation by Pb exposure or reduced representativeness to the target population. In the NAS, cases were ascertained from self-report, which may introduce measurement error.

However, when analyses were restricted to cases recruited from movement disorder clinics and their spouse, in-law, or friend as controls, the results were even stronger (OR: 2.57 [95% CI: 1.11, 5.93] for tibia Pb >13.9 µg/g). Although the use of spouse, in-law, and friend controls can introduce bias, this is expected to be toward the null as these groups are likely to share many exposures. Weisskopf et al. ([2010](#)) did not adjust results for Mn exposure, which has been associated with Parkinsonian symptoms and could potentially confound associations between Pb and Parkinson's disease. However, unlike occupational exposure to Pb, environmental Pb exposure is less likely to be correlated with environmental Mn exposure. Thus, it is less likely that the associations observed with Pb were confounded by co-occurring Mn exposure.

Coon et al. ([2006](#)) conducted a smaller case-control study of 121 Parkinson's disease patients and 414 controls frequency-matched by age, sex, and race, all receiving health care services from the Henry Ford Health System in Michigan. Subjects in the highest quartile of both tibia (OR: 1.62 [95% CI: 0.83, 3.17] for levels ≥ 15 µg/g) and calcaneus (OR: 1.50 [95% CI: 0.75, 3.00] for levels ≥ 25 µg/g) bone Pb levels had higher but imprecise odds of Parkinson's disease compared to those in the lowest quartiles (0-6 µg/g for tibia and 0-12 µg/g for calcaneus). Subjects in the highest quartile of whole-body lifetime Pb level (≥ 81 µg/g, estimated using PBPK modeling) had the highest OR: 2.27 (95% CI: 1.13, 4.55) versus the lowest quartile, 0-40 µg/g. These results were adjusted for age, race, sex, pack-years smoking, regular coffee consumption, and regular alcohol use, but Mn exposure was not considered. It was not clear what the extent of occupational exposure to Pb was among the participants; however, a previous Henry Ford Health System study had linked occupational Pb exposure to Parkinson's disease ([Gorell et al., 1997](#)). Thus, it is uncertain whether the observed associations were confounded by co-occurring Mn exposure.

In summary, a few recent case-control studies expand on previous evidence by finding associations of tibia and calcaneus bone Pb levels, biomarkers of cumulative Pb exposure, with Parkinson's disease in adults. The associations observed with biomarkers of cumulative Pb exposure increase confidence that associations are not explained by reverse causation. However, firm conclusions are not warranted. While associations were adjusted for potential confounding by age, sex, race, and education, Mn co-exposure was not considered.

4.3.9.4 Essential Tremor

The few available case-control studies of essential tremor have found associations with concurrent blood Pb levels. The 2006 Pb AQCD ([U.S. EPA, 2006b](#)) described case-control studies that found associations between concurrent blood Pb levels and essential tremor in New York City metropolitan area populations ([Louis et al., 2005](#); [Louis et al., 2003](#)). In the larger study, mean (SD) blood Pb levels were 3.3 (2.4) µg/dL in the 100 essential tremor cases and 2.6 (1.6) µg/dL in the 143 controls ([Louis et al., 2003](#)). In the other study, mean (SD) blood Pb levels were 3.5 (2.2) µg/dL in the 63 essential tremor cases and 2.6 (1.5) µg/dL in the 101 controls ([Louis et al., 2005](#)). In Louis et al. ([2005](#)), the magnitude of association was larger among the 35 ALAD-2 carriers than among 129 adults homozygous for ALAD-1. History of occupational Pb exposure was similarly rare in cases and controls (2%).

Recently, Dogu et al. ([2007](#)) reported on 105 essential tremor cases selected from a movement disorder clinic in Turkey and 105 controls (69 spouses and 36 other relatives) living in the same district. With adjustment for age, sex, education, smoking status, cigarette pack-years, and alcohol use, a 1 µg/dL higher blood Pb level (measured at the time of study recruitment) was associated with essential tremor with an OR of 4.19 (95% CI: 2.59, 6.78). This OR was much larger than that obtained in the New York area study (OR: 1.19 [95% CI: 1.03, 1.37]) ([Louis et al., 2003](#)). The magnitude of association in Dogu et al. ([2007](#)) is even more striking because so many of the controls were spouses who are expected to share many environmental exposures as cases. Most of the essential tremor cases were retired at the time of the study; however past occupational history was not examined.

In summary, a small body of studies indicates associations between blood Pb level measured at the time of the study and prevalence of essential tremor in adults. However, because of the case-control design, reverse causation cannot be excluded as a potential explanation for the observed associations since loss of physical activity and subsequent bone resorption may lead to an increase in blood Pb level. Further, the level, timing, frequency, and duration of Pb exposure associated with essential tremor are uncertain. History of occupational Pb exposure was not consistently examined, and potential confounding by Mn exposure was not examined.

4.3.9.5 Toxicological Studies of Cell Death Pathways

A common element of the neurodegenerative diseases described above is neuronal cell death. Studies reviewed in the 2006 Pb AQCD ([U.S. EPA, 2006b](#)) documented that Pb exposure induced cell death or apoptosis in various models including rat brain ([Tavakoli-Nezhad et al., 2001](#)), retinal rod cells ([He et al., 2003](#); [He et al., 2000](#)), and cerebellar neurons ([Oberto et al., 1996](#)). Recent studies produced similar findings, in most cases, in animals with higher blood Pb levels than those relevant to this ISA. Long-term (40 days) exposure to 500 ppm Pb in drinking water was found to increase pro-apoptotic Bax protein levels and the number of apoptotic cells in the hippocampus in young (exposure starting at 2-4 weeks of age) and adult (exposure starting at 12-14 weeks of age) male rats with blood Pb levels 91 and 98 µg/dL ([Sharifi et al., 2010](#)). Apoptosis was verified by light and electron microscopy. These investigators found Pb-induced apoptosis of PC12 neuronal cells in vitro ([Sharifi and Mousavi, 2008](#)). Another study followed the developmental profile of changes in various apoptotic factors in specific brain regions of rats exposed to 2,000 ppm Pb acetate during lactation (to PND20) via drinking water of dams ([Chao et al., 2007](#)). At PND20, male offspring blood Pb level was 80 µg/dL. Pb exposure significantly upregulated hippocampal mRNA for various apoptotic factors including caspase-3, Bcl-x, and Brain-derived neurotrophic factor (BDNF) on PND12, PND15 and PND20. The cortex of these male pups showed upregulation of Bcl-x and BDNF on PND20 and PND25. The cerebellum did not have elevated apoptotic mRNA levels in this model. Thus, in this study, Pb-induced apoptosis varied by age and brain region in male offspring.

Pb exposure also has been shown to induce apoptosis of spinal cord cells during spinal cord development in chicks treated with 150 or 450 µg Pb acetate in ovo at embryonic day 3 or 5 and visualized six days later ([Müller et al., 2012](#)). TUNEL positive cells, indicating DNA fragmentation induced by apoptosis, were at significantly higher levels in Pb-exposed animals and were visualized in all layers of the developing spinal cords. Also, levels of glial fibrillary acidic protein (GFAP), a factor important in neuronal migration and cellular differentiation during nervous system development, was significantly attenuated in spinal cords of Pb-exposed chicks. Liu et al. ([2010b](#)) examined apoptotic effects in 30 day-old male rats that were treated with Pb acetate once daily for 6 weeks via intragastric infusion. Four groups: control, low (2 mg/kg BW), medium (20 mg/kg BW), and high (200 mg/kg BW) had blood Pb levels of 1-7.5 µg/dL; 4.5-11.4 µg/dL; 8.9-42 µg/dL; and 48-73 µg/dL, respectively. Pb induced hippocampal neuronal apoptosis (TUNEL positive staining, statistically significant at all Pb doses) and downregulation of hippocampal XIAP (significant at high dose only) and Smac (statistically nonsignificant trend) at the termination of the 6 week treatment. In another study, Pb exposure (500 ppm Pb acetate in drinking water for 8 weeks) of adult male rats

induced regional-specific changes in brain apoptotic proteins poly(ADP-ribose) polymerase, Bcl-2, and caspase-3 with a greater effect observed in the hippocampus and cerebellum than the brainstem or frontal cortex ([Kiran Kumar et al., 2009](#)).

In summary, a small body of epidemiologic studies found Pb-associated increases in essential tremor and Parkinson's Disease in adults. However, the implications of these findings are limited because of the potential for reverse causation to explain cross-sectional associations observed with blood Pb level, and the potential for confounding by Mn exposure. However, toxicological evidence supports an effect of Pb on neurodegeneration by demonstrating that Pb exposure during various lifestages, early postnatal or adulthood, induces neuronal apoptosis in animals. Several of these observations were made with routes (i.e., i.p.) or concentrations of Pb exposure that may not be relevant to this ISA.

4.3.10 Modes of Action for Pb Nervous System Effects

4.3.10.1 Effects on Brain Physiology and Activity

The 2006 Pb AQCD ([U.S. EPA, 2006b](#)) reviewed a small body of available epidemiologic studies demonstrating associations of Pb biomarkers with electrophysiologic and physical changes in the brains of young adults ([Yuan et al., 2006](#); [Cecil et al., 2005](#)) and children ([Meng et al., 2005](#); [Trope et al., 2001](#)) as assessed by magnetic resonance imaging (MRI) or spectroscopy (MRS). The implications of previous findings were limited by the small sample sizes (n = 12-45) and limited consideration for potential confounding. Recent studies examining MRI data were limited largely to the Cincinnati cohort as adults (ages 19-24 years). In addition to supporting associations of childhood blood Pb levels with physiological changes in the brain of adults, these recent analyses expanded on previous studies by including larger sample sizes, aiming to characterize potentially important lifestages of Pb exposures, and evaluating potential links between changes in brain activity and functional neurodevelopmental effects. While overall there are few studies in few populations, by showing physical and physiologic changes in areas of the brain associated with neurodevelopmental function, the evidence provides biological plausibility for the associations observed between Pb biomarker levels and cognitive function and behavior.

In prospective analyses of the Cincinnati cohort as adults (ages 20-23 years, n = 35, 42), Cecil et al. ([2005](#)) and Yuan et al. ([2006](#)) conducted functional MRI during a verb generation language task and found that higher age 3-78 month average blood Pb level (mean 14.2 µg/dL) was associated with decreased activation in the left frontal gyrus and

left middle temporal gyrus, regions implicated in semantic language function. Yuan et al. (2006) considered birth weight, marijuana consumption, sex, SES, gestational age, and IQ as potential confounding factors. Whereas previous analyses of the Cincinnati cohort focused on activity in specific regions of the brain, Cecil et al. (2011) examined brain metabolites. Higher age 3-78 month average blood Pb levels (mean: 13.3 µg/dL) were associated with lower levels of N-acetylaspartate (NAA) and creatine (Cr) in the basal ganglia and lower levels of choline in white matter in 159 adults, ages 19-23 years. These results were adjusted for age and FSIQ; however, several other unspecified factors were considered. Lower levels of NAA, Cr, and choline are linked to decreased neuronal density and alteration in myelin. A recent prospective analysis of 31 men in the NAS cohort similarly reported an association between biomarkers of cumulative, long-term Pb exposure and changes in brain metabolites in older adults. Weisskopf et al. (2007a) found higher tibia and patella Pb levels to be associated with a higher myoinositol/Cr ratio in the hippocampus measured more than 10 years after bone Pb was measured and adjusted for age. Myoinositol/Cr ratio may be indicative of glial activation and is a signal reportedly found in the early stages of HIV-related dementia and Alzheimer's disease.

Other studies in the Cincinnati cohort as young adults found that childhood average blood Pb levels were associated with altered brain architecture. Among 91 adults ages 20-26 years, Brubaker et al. (2009) found associations of age 3-78 month average blood Pb levels (mean: 12.9 µg/dL) with diffusion parameters that were indicative of less organization of fibers throughout white matter. Results were adjusted for maternal IQ, prenatal alcohol and tobacco exposure, and adult marijuana use. In regions of the corona radiata, higher blood Pb levels were associated with less myelination axonal integrity. In regions of the corpus callosum, higher blood Pb levels were associated with greater myelination and axonal integrity. The differential impact among neural elements may be related to the stage of myelination development present at various time periods.

Another study of 157 Cincinnati cohort adults ages 19-24 years provided evidence of region-specific reductions in gray matter volume in association with age 3-78 month average blood Pb levels (mean: 13.3 µg/dL) with adjustment for sex (Cecil et al., 2008). The most affected regions included frontal gray matter, specifically the anterior cingulate cortex, and the ventrolateral prefrontal cortex (i.e., areas related to executive functions, mood regulation, and decision-making). Further, fine motor factor scores were positively correlated with gray matter volume in the cerebellar hemispheres; adding blood Pb level as a variable to the model attenuated this correlation. These findings suggested that changes observed with MRI may mediate the association between blood Pb levels and decrements in motor function. The functional relevance of these structural changes in the brain also is supported by observations from other studies that link changes in brain architecture and activity to changes in cognitive function (e.g., visuoconstruction, visual

memory, eye-hand coordination) ([Schwartz et al., 2007](#)) and behavioral problems (impulsivity, aggression, violence) ([Yang et al., 2005](#); [Raine et al., 2000](#)). In a subsequent comparison of blood Pb levels measured at various lifestages in 157 Cincinnati cohort adults ages 19-24 years, Brubaker et al. ([2010](#)) found that higher blood Pb levels at older ages (annual means from ages 3-6 years, means: 9.6-16.3 $\mu\text{g/dL}$) were associated with greater losses in gray matter volume than were age 1 or 2 year (means: 10.6, 17.2 $\mu\text{g/dL}$), 3-78 month average or maximum blood Pb levels (mean: 23.1 $\mu\text{g/dL}$). Both Cecil et al. ([2008](#)) and Brubaker et al. ([2010](#)) found that Pb-associated reductions in gray matter were more pronounced in males than females in the Cincinnati cohort.

Studies of Pb-exposed workers ($n = 15\text{-}532$) also found associations of concurrent blood (means: 17-63.5 $\mu\text{g/dL}$) and tibia (mean 14.5 $\mu\text{g/dL}$) Pb levels with changes in brain structure and physiology, supporting the effects of chronic Pb exposure. Pb-associated changes included white matter lesions, smaller brain volumes, less total gray matter, and lower levels of brain metabolites such as NAA and Cr ([Hsieh et al., 2009b](#); [Jiang et al., 2008](#); [Bleecker et al., 2007b](#); [Stewart et al., 2006](#)) with adjustment for similar factors as associations for cognitive function. Other occupational exposures were not examined. In a few of these occupational groups, Pb-associated brain changes were linked to poorer performance in cognitive function tests ([Caffo et al., 2008](#); [Bleecker et al., 2007b](#)).

Higher concurrent blood Pb level also was associated with lower NAA/Cr ratio in small cross-sectional studies that included children ($n = 6, 16$, ages 4-21 years), although neither study considered potential confounding ([Meng et al., 2005](#); [Trope et al., 2001](#)). All subjects had normal MRIs with no evidence of structural abnormalities. Thus, the biological relevance of the observed physiological changes is unclear. Additionally, the representativeness of findings is uncertain because results were based on comparisons of subjects with relatively high blood levels (means: 38, 40 $\mu\text{g/dL}$) to those with blood Pb levels <10 $\mu\text{g/dL}$.

In summary, results in a few populations indicate associations of childhood blood or adult tibia Pb levels with changes in brain structure and physiology in adults assessed by MRI or MRS. Associations were found in children, but implications are more limited because of small samples sizes, lack of consideration of potential confounding, and high blood Pb levels of the children examined. Evidence from the prospective Cincinnati cohort studies improves characterization of the temporal sequence between Pb exposure and changes in brain structure and physiology. Several studies linked these changes to functional changes in cognitive performance or motor skills. Because of the small samples sizes of several studies and limited consideration for potential confounding, implications of the effects of Pb exposure specifically on changes in brain structure and physiology are not

certain. However, the evidence provides biological plausibility for the associations observed between Pb biomarker levels and cognitive function and behavioral problems.

4.3.10.2 Oxidative Stress

Because the brain has the highest energy demand and metabolism of any organ, energy homeostasis is of utmost importance. Energy imbalance can increase the susceptibility of the highly energetic brain tissue to stressors and cell death. Pb has been shown to induce energy imbalance by inhibiting various enzymes involved in energy production or glucose metabolism including glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate kinase, and succinate dehydrogenase ([Verma et al., 2005](#); [Yun and Hoyer, 2000](#); [Regunathan and Sundaresan, 1984](#); [Sterling et al., 1982](#)). Mitochondria produce ATP or energy through oxidative phosphorylation. Aberrant mitochondrial function can decrease the energy pool and contribute to ROS formation via electron transport chain disruption. ATP depletion can also affect synaptic and extracellular neurotransmission. The mitochondrial Na^+/K^+ ATPase is important in maintaining the inner mitochondrial membrane potential $\Delta\Psi_m$ (delta psi[sub m]) and the functioning of the mitochondria.

Gestational Pb exposure of mice was found to impair mitochondrial function and energy production in neuronal cells and produce concomitant increases in mitochondrial and cellular ROS production. In these mice, 1,000 ppm Pb acetate in dam drinking water resulted in offspring blood Pb levels of 4 $\mu\text{g}/\text{dL}$ and cerebella Pb levels of 7.2 $\mu\text{g}/\text{g}$ dry weight) ([Baranowska-Bosiacka et al., 2011b](#)). Cerebellar granular cells were harvested from control and Pb-exposed animals at PND8. These neuronal cells were cultured for 5 days in vitro, at which point various mitochondrial parameters were measured. With Pb exposure, ROS were significantly increased in both the cortical granule cells and in the mitochondria. Intracellular ATP concentration and adenylate energy charge values were significantly decreased in cells of Pb-exposed mice versus controls. Neuronal Na^+/K^+ ATPase activity was significantly lower in cortical granule cells from Pb-exposed mice versus cells from controls. Mitochondrial mass was unaffected with Pb treatment, but mitochondrial membrane potential was significantly decreased with Pb exposure. Energy imbalances also were found in Wistar rats (PND15) of each sex injected daily for 2 weeks with Pb acetate (15mg/kg BW, i.p., resulting in a mean blood Pb level of 31 $\mu\text{g}/\text{dL}$; control blood Pb level 3 $\mu\text{g}/\text{dL}$) ([Baranowska-Bosiacka et al., 2011a](#)). ATP and ADP were significantly decreased in various brain regions with Pb exposure, with the cerebellum and hippocampus more strongly affected than the forebrain cortex. Also, expression of the pro-inflammatory P2XR receptor was enhanced in the glial fraction, indicating the astrocyte pool may be involved in the pathological changes found in Pb-exposed immature rat brains. Mitochondrial energy imbalances also were found in

Pb-exposed crayfish that were placed under hypoxic conditions which induced a decrease in metabolism ([Morris et al., 2005](#)).

In rats, Pb exposure has been shown to induce oxidative stress, in some cases, with concomitant functional CNS changes. Exposure of adult male rats to 4,000 ppm Pb acetate in drinking water for 6 weeks increased brain levels of lipid peroxides (LPO) and lowered levels of antioxidants including nitric oxide (NO), total antioxidant capacity (TAC), glutathione (GSH), glutathione-S-transferase (GST), and superoxide dismutase (SOD). In red blood cells, Pb levels were positively correlated with LPO levels and negatively correlated with NO levels ([Hamed et al., 2010](#)). These effects of Pb on oxidative stress parameters were attenuated with co-exposure to green tea extract (1.5%), which reduced brain (1.9 to 1.2 ppm) and blood Pb levels of rats (0.773 to 0.654 ppm). In a study of adult male Wistar albino rats, Pb acetate treatment by i.p. (20 mg/kg daily, 5 days) elevated lipid peroxidation, neuronal damage, and brain tissue DNA fragmentation and decreased antioxidant GSH levels and antioxidant enzyme activity, ([Abdel Moneim et al., 2011a](#)). These effects were attenuated with co-administration of the polyunsaturated fatty acid flaxseed oil (oral gavage 1,000mg/kg body weight for 5 days, 1 hour prior to Pb dosing). Flaxseed oil co-treatment also was associated with significantly lower blood Pb level in Pb-exposed animals and control animals, indicating that flaxseed oil may alter Pb toxicokinetics in animals. Another study provided indirect evidence of Pb-induced oxidative stress with observations that Pb-induced (2,000 ppm Pb acetate in drinking water PND1-PND67) impairments in long-term potentiation (LTP), paired-pulse reactions, and input/output functions in the DG of male and female Wistar rats were significantly attenuated with co-treatment with the antioxidant quercetin (i.p. 30 mg/kg BW, PND60-PND67) at PND67 ([Hu et al., 2008a](#)). Quercetin-treated animals had significantly less hippocampal Pb than did the animals exposed only to Pb.

Oxidative stress may be involved in neurodegenerative pathologies including Alzheimer's disease. Hydrogen peroxide-induced oxidative stress has been shown to induce intracellular accumulation of Ab in human neuroblastoma cells ([Misonou et al., 2000](#)).

Oxidative stress-induced DNA damage can be measured as the ratio of the adduct 8-hydroxy-2'-deoxyguanosine to 2-deoxyguanosine (8-oxo-dG/2-dG). 2-dG is a product of oxidative cleavage and is oxidized to form 8-oxo-dG. Pb-induced changes in the 8-oxo-dG to 2-dG ratio were examined recently as a mechanism underlying neurodegeneration. Similar to Ab levels, changes in the 8-oxo-dG to 2-dG ratio showed a biphasic relationship in the brains of rats exposed to 2,000 ppm Pb acetate via drinking water of dams from PND1-PND20 (blood Pb level 46 µg/dL) ([Bolin et al., 2006](#)). The 8-oxo-dG to 2-dG ratio decreased early in exposure (PND5) but increased at age 20 months. No increase was found in animals exposed to Pb from age 18 to 20 months (blood Pb level: 60 µg/dL). Activity of the base-excision DNA repair enzyme 8-oxo-

guanine DNA glycosylase was unaffected by Pb exposure. Similar findings were reported in a monkey study ([Wu et al., 2008a](#)). The ratio of 8-oxo-dG to 2-dG in the brains of aged monkeys (23 years) was significantly elevated above that in controls with Pb exposure only in infancy (PND1-PND400, infant formula, blood Pb levels: 19-26 µg/dL at PND400) ([Wu et al., 2008a](#)). Thus, evidence in rats and monkeys suggests a possible role for oxidative stress in Pb-induced neurodegenerative effects and indicates that early life Pb exposure induces oxidative DNA damage and amyloidogenesis in aged animals.

4.3.10.3 Nitrosative Signaling and Nitrosative Stress

The NO system is increasingly being recognized as a signaling system in addition to its more classical role as a marker of cellular stress. Pb exposures during the gestational-early postnatal period (GD6-PND21) ([Chetty et al., 2001](#)) and after lactation only ([Fan et al., 2009a](#)) were found to reduce hippocampal levels of NO or neuronal NO synthase. In the hippocampus, NO mediates LTP, which is considered to be a major cellular mechanism underlying learning and memory. Thus, observations of Pb-induced changes in hippocampal NO may provide a mechanistic explanation for the effects of Pb on cognitive function decrements. Fan et al. ([2009a](#)) found reduced hippocampal NOS and NO in weanling male rats after either 4 or 8 weeks of Pb exposure resulting in blood Pb levels of 48 and 70 µg/dL, respectively. In the same study, dietary supplementation with taurine or glycine concomitant with 8 weeks of Pb exposure induced significant increases in hippocampal NOS, whereas Pb plus dietary supplementation with vitamin C, methionine, tyrosine, or vitamin B1 decreased hippocampal NOS. In this study, co-exposure of specific nutrients also prevented Pb-induced impairments in learning as evidenced by lack of increased escape latency in the Morris water maze. Dietary supplementation with tyrosine, methionine, or ascorbic acid after 4 weeks of Pb exposure (blood Pb levels upon cessation of exposure and after 4-week lag: 48 and 8.1 µg/dL, respectively) reversed Pb-induced decrements in NO/NOS. Zn supplementation given after Pb exposure had no effect on the NO system.

4.3.10.4 Synaptic Changes

Previous toxicological studies pointed to an effect of developmental Pb exposure on synapse development, which mechanistically may contribute to multiple Pb-related aberrant effects, including changes in LTP and facilitation. Facilitation of a neuronal terminal is defined as the increased capability to transmit an impulse down a nerve due to prior excitation of the nerve. Earlier work showed that Pb exposure resulted in altered density of dendritic hippocampal spines ([Király and Jones, 1982](#); [Petit and Leboutillier,](#)

1979), reduced synapse formation ([Chen et al., 2005b](#)), and abnormal long-term plasticity ([Nihei et al., 2000](#)). In a recent study, Li et al. ([2009c](#)) focused on inflammatory endpoints and synaptic changes after gestational plus lactational Pb exposure (1,000-10,000 ppm Pb acetate via drinking water of dams, producing offspring blood Pb levels 40-100 µg/dL, respectively at PND21). Hippocampal TNF-α was significantly elevated with Pb exposure, and proteins that comprise the SNARE complex were all changed with Pb exposure. The SNARE complex of synaptic proteins includes SNAP-25, VAMP-2 and Syntaxin 1a and is essential in exocytotic neurotransmitter release at the synapse. Thus, Li et al. ([2009c](#)) found significant differences in hippocampal synaptic protein composition and increased pro-inflammatory cytokine levels in the brains of Pb-exposed offspring.

Recent research using the *Drosophila* larval neuromuscular junction model showed that compared with unexposed controls, Pb-exposed larvae had significant increases in intracellular calcium and significant delays in calcium decays back to baseline levels at the pre-synaptic neuronal bouton (as stimulated with multiple action potentials, also called AP trains). Pb-exposed larvae had reduced activity of the plasma membrane Ca²⁺ATPase, which is responsible for extravasations of calcium from the synaptic terminal ([He et al., 2009](#)). Intracellular calcium in Pb-exposed larvae was no different from that in controls under resting conditions or in neurons with stimulation by a single action potential. Pb media concentrations in these experiments were 100 or 250 µM with the body burden of Pb from the lower dose estimated to be 13-48 µM per larvae. After stimulation of the axon, facilitation of the excitatory post-synaptic potential, which is dependent on residual terminal calcium, was significantly elevated in Pb-exposed larvae versus control ([He et al., 2009](#)). The data from this synapse study demonstrate that developmental Pb exposure affected the plasma membrane Ca²⁺ATPase, induced changes in the intracellular calcium levels during impulse activation, and produced changes in facilitation of the neuronal networks of *Drosophila*. Thus, the neuromuscular junction is a potential site of Pb interaction.

Neurotransmission is an energy-dependent process as indicated by the presence of calcium-dependent ATP releases at the synaptic cleft. At the synapse, ATP is metabolized by ectonucleotidases. Acute exposure (96 hours) of male and female zebrafish to 2 µg/dL Pb acetate in their water induced significant decreases in ATP hydrolysis in brain tissue ([Senger et al., 2006](#)). This dose is deemed to be environmentally relevant. With chronic exposure (30 days), Pb acetate promoted the inhibition of ATP, ADP and AMP hydrolysis. These findings were consistent with findings in rodents ([Baranowska-Bosiacka et al., 2011a](#)). The authors hypothesized that at 30 days, this Pb-induced change in nucleotide hydrolysis was likely due to post-translational modification because expression of enzymes responsible for the hydrolysis,

NTPDase1 and 5'-nucleotidase, were unchanged ([Senger et al., 2006](#)). Thus, Pb was shown to affect nucleotidase activity in the CNS of zebrafish, possibly contributing to aberrant neurotransmission.

Another enzyme important in synaptic transmission at cholinergic junctions in the CNS and at neuromuscular junctions is acetylcholinesterase (AChE). After 24 hours of exposure to 2 µg/dL Pb acetate in water, AChE activity was significantly inhibited in zebrafish brain tissue ([Richetti et al., 2010](#)). AChE activity returned to baseline by 96 hours and maintained baseline activity after 30 days of exposure. Thus, Pb was shown to affect synaptic homeostasis of AChE in the brains of zebrafish only transiently.

Pb has been shown to act as an antagonist of the NMDA receptor (NMDAR). The NMDAR is essential for proper presynaptic neuronal activity and function. Primary cultures of mouse hippocampal cells exposed to 10 or 100 µM Pb during the period of synaptogenesis had loss of two proteins necessary for presynaptic vesicular release, synaptophysin (Syn) and synaptobrevin (Syb) but no change in a similar protein synaptotagmin (Syt) ([Neal et al., 2010a](#)). This deficit was found in both GABAergic and glutamatergic neurons. Pb also induced an increase in the number of presynaptic contact sites. But, these sites may have been nonfunctional as they lacked the protein receptor complexes necessary for proper vesicular exocytosis. Another factor involved in maturation and signaling of presynaptic neurons is BDNF, which is synthesized and released by postsynaptic neurons and regulated by the NMDAR. In mouse hippocampal cells, both pro-BDNF and BDNF release were significantly attenuated with Pb exposure ([Neal et al., 2010a](#)). Further, exogenous BDNF administration rescued the aforementioned Pb-related effects on presynaptic proteins. Thus, results from this cell culture model showed that Pb-related presynaptic aberrations are controlled by NMDAR-dependent BDNF effects on synaptic transmission.

Glutamate is another neurotransmitter that is released from presynaptic neurons and via interactions with the NMDAR causes postsynaptic neuron depolarization. A recent study of Wistar albino rats exposed to Pb postnatally from birth to age 12 weeks (drinking water 3×10^4 µg/dL Pb acetate, resulting in blood Pb levels of 17 µg/dL at age 6 weeks) showed decreased learning ability, decreased hippocampal glutamate at 6, 8, 10 and 12 weeks of age, as well as significant decrements in the hippocampal glutamate synthesis-related enzymes aspartate aminotransferase and alanine aminotransferase ([Niu et al., 2009](#)).

4.3.10.5 Blood Brain Barrier

Two barrier systems exist in the body to separate the brain or the CNS from the blood: the blood brain barrier (BBB) and the blood cerebrospinal fluid barrier (BCB). The BBB, formed by tight junctions at endothelial capillaries forming the zonulae occludens (occludins, claudins, and cytoplasmic proteins), separates the brain from the blood and its oncotic and osmotic forces, allowing for selective transport of materials across the BBB.

Pb exposure during various developmental windows has been shown to increase the permeability of the BBB of animals ([Dyatlov et al., 1998](#); [Struzynska et al., 1997b](#); [Moorhouse et al., 1988](#); [Sundstrom et al., 1985](#)). Possibly due the underdevelopment of the BBB early in life, prenatal and perinatal Pb exposure has been found to result in higher brain Pb accumulation than have similar exposures later in life ([Moorhouse et al., 1988](#)). The choroid plexus and cerebral endothelial cells that form the BBB and BCB tight junctions have been shown to accumulate Pb more than other cell types and regions of the CNS. Studies reviewed in the 2006 Pb AQCD showed that the chemical form of Pb and its capability to interact with proteins and other blood components affect its capability to penetrate the BBB ([U.S. EPA, 2006b](#)). Pb also has been shown to compromise the BCB and decrease the cerebrospinal fluid level of transthyretin, which binds thyroid hormone in the cerebrospinal fluid. Low thyroid hormone levels in pregnant women have been linked with IQ deficits in their children ([Lazarus, 2005](#)).

Recent research with male weanling rats exposed to Pb acetate via drinking water showed leaky cerebral vasculature, an indication of a compromised BBB, as detected histologically with lanthanum nitrate staining of the brain parenchyma ([Wang et al., 2007b](#)). Cerebral vasculature leakiness was ameliorated or resembled that of controls after Fe supplementation. The cerebral vasculature leakiness may be explained by observations of significant Pb-induced decreases in the BBB tight junction protein occludin in the hippocampus, brain cortex, and cerebellum of these weanling animals. Occludin levels were rescued to control levels with Fe supplementation. This loss of integrity at the junctional protein level was affirmed with additional experiments using the rat brain vascular endothelial cell line RBE4, in which 10 μ M Pb acetate exposure for 2, 4, 8, 16 and 24 hours resulted in decreases in junctional proteins occludin and claudin 5 as well as scaffold proteins ZO1 and ZO2 ([Balbuena et al., 2011](#)). Because gene expression for these junctional and scaffold proteins did not show decrements, it was determined that these protein decrements were due to post-translational modifications.

Pb exposure also was found to contribute to leakiness of the BBB by decreasing the resistance across the junction ([Balbuena et al., 2010](#)). An in vitro co-culture system employing endothelial cells (RBE4 or bovine brain microvascular endothelial cells) and astrocytes (primary Sprague-Dawley neonatal pup astrocytes, GD21) served as the barrier

between Pb-containing media and neurons. Pb acetate exposure (1 and 10 μ M) for 14 hours significantly impaired transendothelial electrical resistance, a marker of BBB integrity, in a concentration-dependent manner.

Long-term Pb exposure of adult mice was found to increase regional edema and BBB permeability ([López-Larrubia and Cauli, 2011](#)). Adult male rats exposed to Pb acetate in drinking water for 4 or 12 weeks (50 or 500 ppm, resulting in blood Pb levels of 12 and 55 μ g/dL, respectively) were assessed by diffusion weighted imaging for changes in apparent diffusion coefficient (ADC), a measure of tissue water diffusivity that changes under pathological conditions like cerebral edema. With 12 weeks of exposure, 50 ppm Pb increased the ADC values in the cerebellum and mesencephalic reticular formation, and 500 ppm Pb exposure significantly increased ADC in the corpus callosum and caudate putamen. With 4 weeks of exposure, 500 ppm Pb significantly increased the water ADC in the hippocampus, mesencephalic reticular formation, and cerebellum but not in other brain areas. The brain areas with elevated ADC also showed increased BBB permeability as measured with evans blue dye.

4.3.10.6 Cell Adhesion Molecules

Classic cell adhesion molecules including neural cell adhesion molecule (NCAM) and the cadherins are junctional or cell surface proteins that are critical for cell recognition and adhesion. While direct effects of Pb on cell adhesion molecules have not been described, the calcium-dependency of these molecules suggests possible interactions with competing cations like Pb and a possible contribution to Pb effects on CNS barrier function disruption, neurite outgrowth, synaptic plasticity, learning, and memory ([Prozialeck et al., 2002](#)).

4.3.10.7 Effects on Glial Cells

Astroglia and oligodendroglia are supporting cells in the nervous system that maintain the extracellular space in the brain and provide structural support to neurons, deliver nutrients to neurons, and promote myelination. Glial cells provide immune surveillance in the brain and contribute to inflammation-mediated pathologies. In Wistar rats, Pb treatment (15 mg/kg of Pb acetate, i.p.) during early postnatal maturation was observed to produce chronic glial activation with inflammation and neurodegeneration ([Struzyńska et al., 2007](#)). Among the cytokines detected in the brains of these Pb-treated rats were IL-1 β , TNF- α and IL-6. Glial cells have been shown to serve as Pb sinks in the developing and mature brain by sequestering Pb ([Tiffany-Castiglioni et al., 1989](#)). This

glial sequestration of Pb has been accompanied by a decrease in brain glutamine and a reduction in glutamine synthetase activity in the astroglia; astroglia take up released glutamate and convert it to glutamine. Pb has been shown to induce hypomyelination and demyelination ([Coria et al., 1984](#)) mediated through the oligodendrocytes with younger animals found to be more susceptible to the effects of Pb ([Tiffany-Castiglioni et al., 1989](#)). Pb accumulation in young glial cells may contribute to a lifelong exposure of neurons to Pb as Pb is released from the sink over time. Thus, Pb accumulation in glial cells can contribute to continual damage of surrounding neurons ([Holtzman et al., 1987](#)).

Glial transmitters

Evidence indicates that glial transmission is affected with Pb exposure and that the NMDAR may be involved in this aberrant glial transmission. To determine the contribution of the gliotransmitter serine to Pb-mediated changes in LTP, Sun et al. ([2007](#)) exposed rats to Pb acetate from gestation through lactation to PND28 via maternal drinking water and collected hippocampal sections. CA1 section LTPs were examined using in vitro patch clamp monitoring. Chronic Pb exposure impaired the magnitude of hippocampal NMDAR-dependent LTPs, but the magnitude of the LTPs was restored with supplementation with D-serine ([Sun et al., 2007](#)), which is an NMDAR agonist ([Bear and Malenka, 1994](#)). The use of 7-chlorokynurenic acid, an antagonist of the glycine binding site of the NMDAR, which also is the binding site of D-serine, effectively abolished the rescue of LTP by D-serine. NMDAR-independent LTP hippocampal neurotransmission was inhibited in slices of Pb-exposed mossy-CA3 synapses and was not rescued by exogenous D-serine supplementation.

4.3.10.8 Neurotransmitters

Pb has been shown to compete with calcium for common binding sites and second messenger activation. When Pb activates a calcium-dependent system in the nervous system, it can contribute to aberrant neurotransmitter regulation and release because this system intimately relies on calcium signaling for its homeostasis. Pb also has been shown to interfere with other physiological divalent cations. Pb-related alterations in neurotransmission are discussed in further detail below.

Monoamine Neurotransmitters and Stress

The monoamine neurotransmitters include dopamine (DA), serotonin (5HT), and norepinephrine (NE). Combined exposures of maternal stress and Pb exposure can

synergistically enhance neurobehavioral impairments in offspring and can sometimes potentiate an effect that would otherwise be sub-threshold. Virgolini et al. ([2008a](#)) found enhanced DA and NE release in male rats and enhanced NE release in female rats after developmental Pb exposure (50 or 150 ppm in drinking water, 2 months prior to mating through lactation, resulting in blood Pb levels of 11 µg/dL and 35 µg/dL, respectively) and combined maternal and offspring stress. In most cases, stress potentiated the effects of Pb exposure on offspring NE and DA concentrations. Regional 5HT levels were unaffected in offspring with Pb exposure alone. Pb (50 and 150 ppm) combined with stress (maternal and/or offspring) significantly potentiated 5HT levels in the frontal cortex in females and in the nucleus accumbens (NAC) and striatum in male offspring. The concentration of 5-Hydroxyindoleacetic acid (5HIAA), the main metabolite of 5HT, was significantly increased in the striatum of male offspring with 150 ppm Pb exposure alone. With 50 ppm Pb, stress potentiated striatal and frontal cortex 5HIAA in males. Potentiation of 5HIAA levels in females was significant in the NAC with 50 ppm Pb exposure; stress alone also significantly increased 5HIAA levels in the NAC of females with no Pb exposure. Pb-induced changes in brain neurochemistry with or without concomitant stress exposure are complex with differences varying by brain region, neurotransmitter type, and sex of the animal.

Monoamine Neurotransmitters and Auditory Function

Earlier work showed that perinatal Pb exposure of rats induced increased tyrosine hydroxylase, DA, and cerebral cortex catecholamine neurotransmission ([Devi et al., 2005](#); [Leret et al., 2002](#); [Bielarczyk et al., 1996](#)). Earlier publications examining various time windows, durations, and doses of Pb exposure indicated varying effects on monoamine neurotransmitters. In recent work, these neurotransmitters, among others, have been implicated in Pb effects on auditory function in various integration centers of the brainstem including the lateral superior olive (LSO) and the superior olivary complex (SOC). Among various functions, the SOC is vital for sound detection in noisy settings. A recent study in mice found significant decreases in immunostaining of LSO and SOC brainstem sections for monoamine vesicular transporter VMAT2, 5HT, and dopamine beta-hydroxylase (DbH, a marker for NE) after gestational-lactational Pb exposure (10 or 100 µM Pb acetate from the formation of breeding pairs to PND21) ([Fortune and Lurie, 2009](#)). This exposure period corresponds to the period of auditory development in the mouse. Statistically significant decreases in VMAT2 and DbH were found in mice with blood Pb levels of 8.0 and 42 µg/dL; however, decrements in 5HT were statistically significant only in mice with 8.0 µg/dL blood Pb level. Immunostaining for tyrosine hydroxylase and transporters including VGLUT1, VGAT, VACHAT indicated that they were unaffected by developmental Pb exposure. These data provide evidence that specific

regions of the brainstem involved in auditory integration are affected by developmental Pb exposure via effects on monoamine neurotransmitters. The Pb-induced effects on the monoamine system of the portion of the brainstem involved in auditory function provide possible mechanistic explanation for the epidemiologic and toxicological evidence for Pb-associated decrements in auditory processing ([Section 4.3.6.1](#)).

Dopamine

The 2006 Pb AQCD ([U.S. EPA, 2006b](#)) detailed evidence for Pb-related decreased dopaminergic cell activity in the substantia nigra and ventral segmental areas. Earlier studies with postnatal or adult Pb exposure reported changes in DA metabolism, as indicated by changes in DA and DOPAC, a DA metabolite. Expanding upon these findings, a recent study assessed DA metabolism in various brain regions of year-old male C57BL/6 mice after gestational plus lactational Pb exposure ([Leasure et al., 2008](#)). Both 27 and 109 ppm Pb acetate induced significant elevations in DOPAC concentration and the DOPAC to DA ratio in the forebrain. In the forebrain, DA was significantly decreased with 27 ppm Pb and significantly elevated with 109 ppm Pb compared to controls. In the striatum, DOPAC was significantly elevated with both doses, but DA was significantly elevated only with the higher dose. The striatum ratio of DOPAC to DA was not significantly different from that in controls. These recent data expand upon the monoamine literature base which indicates that Pb exposure of rats during the gestational/lactational, lactational, or postweaning period producing blood Pb levels 9-34 µg/dL increases sensitivity of the dopamine receptors (D2 and D3) ([Gedeon et al., 2001](#); [Cory-Slechta et al., 1992](#)), produces higher DA levels ([Devi et al., 2005](#); [Leret et al., 2002](#)), and enhances catecholamine neurotransmission in the cerebral cortex, cerebellum, and hippocampus ([Devi et al., 2005](#)).

The interaction of DA and the NO system in the striatum was studied after prenatal Pb exposure ([Nowak et al., 2008](#)). Blood Pb levels were not reported in this study, but similarly treated Wistar rat pups had blood Pb levels at parturition in range of 50-100 µg/dL ([Grant et al., 1980](#)). 7-nitroindazole (7-NI), a selective inhibitor of nNOS, enhanced amphetamine-evoked DA release in the rat striatum ([Nowak et al., 2008](#)). Prenatal Pb exposure attenuated the facilitatory effect of 7-NI on DA release in the striatum. This interaction was ROS-independent; using spin trap measurements, investigators found no significant concentration changes in hydroxyl radical with Pb exposure ([Nowak et al., 2008](#)). Thus, the neuronal NO system appears to be involved in certain aspects of Pb-related dopaminergic changes.

In various animal models, the loss of retinal DA, dopamine turnover (DOPAC:DA ratio), or Zn was associated with Pb-induced abnormal rod-mediated scotopic ERGs. These

effects may explain observations of Pb-associated subnormal or supernormal ERGs observed in animals and children ([Rothenberg et al., 2002b](#); [Lilienthal et al., 1994](#); [Lilienthal et al., 1988](#); [Alexander and Fishman, 1984](#)) ([Section 4.3.6.2](#)), although the biological relevance of the variable effects of Pb exposure on subnormal versus supernormal ERGs is not clear.

NMDA Receptors

The glutamate receptor, NMDAR, has been shown to contribute to synaptic plasticity, and Pb exposure at various developmental stages has been shown to contribute to aberrations in LTP or LTD in the hippocampus via reduced NMDA current, among other mechanisms ([Liu et al., 2004](#)). The 2006 Pb AQCD ([U.S. EPA, 2006b](#)) indicated that Pb attenuated the stimulation of glutamate release, which in turn, affected LTP. Further, the effects of Pb exposure on decreasing the magnitude of LTP and increasing the threshold of the LTP in the hippocampus were found to be biphasic or nonlinear. NMDAR subtypes have been shown to be significantly decreased with developmental Pb exposure ([Guilarte and McGlothlan, 1998](#)). Recent evidence indicated Pb-related decreases in the gene expression and protein level of NMDAR subunits NR1, NR2A, and NR2B in weanling male rats exposed to 4×10^4 µg/dL Pb acetate in drinking water for 8 weeks. Several of these responses were attenuated with methionine-choline co-exposure ([Fan et al., 2010](#)). Other recent mechanistic studies found that pretreatment of primary fetal brain neuronal rat cultures with glutamic acid, a NMDAR agonist, reversed Pb-induced reductions in NMDAR subunits ([Xu and Rajanna, 2006](#)) whereas pretreatment with the NMDA antagonist MK-801 exacerbated Pb-induced NMDAR deficits ([Xu and Rajanna, 2006](#)). Further strengthening the link among Pb exposure, NMDAR function, and learning, Guilarte et al. ([2003](#)) demonstrated that rats exposed to 1,500 ppm Pb during gestation and lactation then reared in isolation, had reduced expression of hippocampal NR1, reduced induction of BDNF mRNA, and learning impairment. These effects were attenuated in Pb-exposed rats reared in an enriched environment with toys.

Other Glutamate Receptors

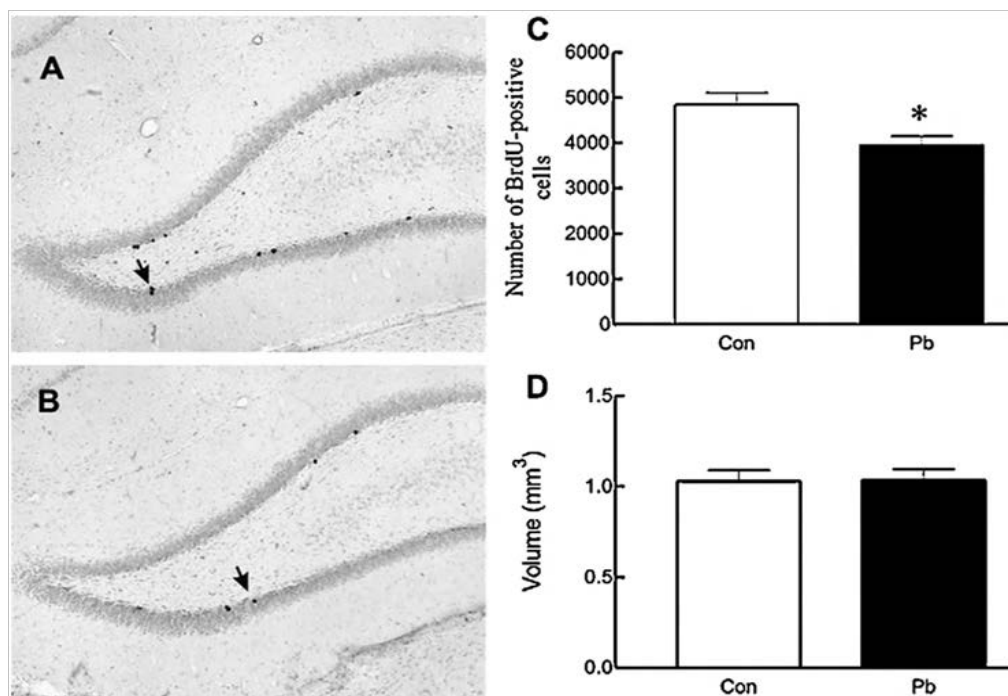
The metabotropic glutamate receptor (mGluR) is another well-recognized target of Pb toxicity. In vitro (GD18 fetal rat hippocampal neurons, 0.01, 100, 1 µM Pb chloride in culture media) and in vivo experiments (gestational-lactational Pb acetate exposure; 500, 2,000, 5,000 ppm in dam drinking water, with respective weanling rat blood Pb levels of 18, 57, 186 µg/dL) showed that Pb exposure decreased mGluR5 mRNA and protein in a concentration-dependent manner ([Xu et al., 2009c](#)). Recent evidence indicates a role for

mGluR5 in synaptic plasticity, LTP, and LTD; thus, the Pb-related attenuation of mGlu5 expression may represent a mechanism by which Pb impairs learning and memory.

4.3.10.9 Neurogenesis

Studies continued to show that Pb exposure decreases neurogenesis (i.e., proliferation of neuronal cells) in the hippocampus, which is important in LTP, spatial learning, neuronal outgrowth, and possibly effects related to schizophrenia pathophysiology. Coherence for these findings is provided by evidence for Pb-induced decreases in NMDAR, which mediates the integration of new neurons into existing neuronal pathways in the adult hippocampal DG. Earlier work by Schneider et al. ([2005](#)) showed that postnatal Pb exposure (PND25 to PND50 or PND55, 1,500 ppm Pb acetate in chow, resulting in blood Pb level of 20 µg/dL) of male Lewis rats induced significant decrements in BrdU incorporation (an indicator of DNA replication) at PND50-PND55.

Recent publications affirm this previous finding with different sex of animals, dosing and exposure time windows. Postnatal Pb exposure of Wistar rat pups from PND1 to PND30 (2,000 ppm Pb acetate, resulting in blood Pb levels of 34 and 6.5 µg/dL at PND21 and PND80, respectively) induced a statistically significant decrement in the number of new cells (BrdU positive cells) in the DG at PND80 ([Fox et al., 2010](#)) ([Figure 4-13](#)).



Note: Light micrograph pictures of BrdU positive cells (proliferating cells undergoing DNA replication) shown as black dots, show more proliferating cells in control (A) than Pb-exposed rats (B). Counts of BrdU positive cells (C) but not Volume of hippocampus dentate gyrus (D), are lower in Pb-exposed rats (black bars) than control (white bars) rats. *p < 0.05 vs. control. Rats were exposed to 2,000 ppm Pb acetate postnatal day 1-30 (blood Pb levels 34 µg/dL) and were examined at postnatal day 80.

Source: Reprinted with permission of Elsevier Science, Fox et al. (2010)

Figure 4-13 Neurogenesis (production of new cells) in the rat hippocampal dentate gyrus after early postnatal Pb exposure.

In another study, lifetime Pb exposure beginning in gestation (1,500 ppm Pb acetate in chow from 10 days before mating to PND50 or PND78, resulting in blood Pb levels 26 µg/dL) induced significant decrements in hippocampal granule cell neurogenesis in adult Long-Evans rats (Verina et al., 2007). Also, Pb-exposed animals had significant decreases in brain volume in the stratum oriens (SO) region of the hippocampus, specifically in the mossy fiber terminals of the SO. Pb-exposed animals also showed a significant decrease in the length-density of immature or newly-formed neurons in the outer portion of the DG. These findings show that Pb exposure at doses relevant to this ISA induced significant decreases in adult hippocampus granule cell neurogenesis and morphology, potentially providing mechanistic explanations for Pb-induced neuronal aberrations and impairments in downstream effects such as learning and memory. Exposure of zebrafish embryos to Pb (50–700 µM Pb acetate in embryo medium from 0 to 6 days post hatch) caused significant apoptosis of brain cells (increased TUNEL

positive brain cells) and decreased brain levels of some (gfap and huC) but not all (crestin and neurogenin1) genes involved in neurogenesis ([Dou and Zhang, 2011](#)).

4.3.10.10 Neurite Outgrowth

As described in the 2006 Pb AQCD ([U.S. EPA, 2006b](#)), Pb was shown to decrease neurite outgrowth in vitro and mediate such effects via protein kinase mediated pathways (MAPK/ERK); earlier work had documented decreased primary DA neuron outgrowth with 0.001 μ M Pb exposure in vitro ([Schneider et al., 2003](#)). A recent study showed that gestational exposure of female Wistar rats to 500-4,000 μ M Pb chloride (resulting in offspring blood Pb levels up to 12 μ g/dL) significantly decreased offspring hippocampal neurite outgrowth and reduced the expression of hippocampal polysialylated neural cell adhesion molecule (PSA-NCAM), NCAM, and sialyltransferase ([Hu et al., 2008b](#)). PSA-NCAM is transiently expressed in newly formed neurons during the period of neurite outgrowth from embryogenesis until the early postnatal period and is down-regulated in the brains of adults except in areas known to exhibit synaptic plasticity ([Seki and Arai, 1993](#)). NCAM is important for memory formation, plasticity, and synapse formation, and its suppression by gestational Pb exposure may represent a mechanism that mediates Pb-associated impairments in cognitive function.

4.3.10.11 Epigenetics

Many investigators are beginning to show that environmental chemical exposures and air pollution exposure are associated with epigenetic changes in humans ([Baccarelli and Bollati, 2009](#); [Pavanello et al., 2009](#); [Tarantini et al., 2009](#); [Bollati et al., 2007](#)).

Epigenetic changes involve changes in DNA expression without changes in the DNA sequence, and these changes may be heritable. Epigenetic changes include changes in histone modification, DNA methylation, miRNA, or pathways that affect these processes. Differential epigenetic modification has the potential to contribute to disease by silencing or activating genes in an aberrant manner. For example, a recent study identified differential methylation of a specific locus in monozygotic twins discordant for schizophrenia ([Dempster et al., 2011](#)); Pb was not examined in this study.

DNA methyltransferases catalyze the transfer of a methyl group to DNA and are important in epigenetics (i.e., silencing of genes like tumor suppressors) and imprinting. DNA methyltransferase activity was significantly decreased in cortical neurons from aged monkeys at ages 20-23 years after infancy exposure (1.5 mg/kg Pb acetate via diet per day PND1-PND400, blood Pb level 19-26 μ g/dL at PND400) and in fetal mouse

brain cells 1 week after 24-hour exposure to 0.1 μ M Pb in culture ([Wu et al., 2008a](#)). Cerebral cortex tissue was obtained from female monkeys examined in this study for further analysis. Decreases in DNA methyltransferases (Dnmt1, Dnmt3a) were noted in control monkey brains as they aged, and these decreases were exacerbated by infancy Pb exposure ([Bihaqi et al., 2011](#)). Another enzyme involved in DNA methylation, methyl CpG binding protein 2, showed a similar trend as the Dnmts. Profiles of the histone modifying proteins increased with age in control animals. These age-related increases were significantly attenuated in Pb-exposed animals. Methyltransferases catalyze biological methylation reactions using cofactor S-adenosyl methionine (SAM) as the methyl donor. In rats, SAM exposure after gestational-lactational Pb exposure (1,500 ppm Pb acetate via drinking water of dams followed by 20-22 days of daily 20 mg/kg BW SAM exposure of offspring) improved hippocampal LTP and Morris water maze performance at PND44-PND54 ([Cao et al., 2008](#)). Thus, the improved cognition and synaptic plasticity observed with co-exposure to Pb and the methyl donor SAM suggest that methylation reactions may be involved in Pb-associated effects on cognition.

4.3.10.12 Cholesterol and Lipid Homeostasis

Various pathological conditions are associated with elevated plasma free fatty acids or elevated cholesterol. Adult male rats exposed to Pb acetate (200, 300, or 400 ppm) in their drinking water for 12 weeks had increased cholesterologenesis and phospholipidosis in brain tissue ([Ademuyiwa et al., 2009](#)). Pb-induced changes in brain cholesterol showed an inverse U concentration-response relationship, with the largest increase in brain cholesterol observed with 200 ppm Pb followed by 300 ppm Pb. Animals exposed to 400 ppm Pb did not have significant changes in brain cholesterol. In a separate study, Pb treatment (single dose 100 μ mol/kg, i.v.) was shown to depress the activity of cholesterol-7- α -hydroxylase, an enzyme involved in biosynthesis of bile acid, which mediates elimination of cholesterol from the body ([Kojima et al., 2005](#)). In Ademuyiwa et al. (2009), Pb exposure significantly increased brain triglycerides by 83% at 300 ppm and by 108% at 400 ppm. At 200 ppm, Pb exposure induced a statistically nonsignificant decrease in brain triglycerides. All three Pb concentrations induced significantly increased brain phospholipids. Interestingly, plasma free fatty acids were significantly elevated in a concentration-dependent manner; plasma triglycerides and cholesterol were unaffected by Pb exposure. The molar ratio of brain cholesterol to phospholipids, an indicator of membrane fluidity, was significantly increased at 200 and 300 ppm Pb exposure, indicating increased membrane fluidity. Brain Pb in all dose groups was below the limit of detection (0.1 ppm). Blood Pb levels for the 0; 200; 300; and 400 ppm Pb exposures were 7; 41; 61; and 39 μ g/dL, respectively; higher than those relevant to this

ISA. In summary, a recent study found that adult 12-week Pb exposure significantly increased brain cholesterol, triglycerides, and phospholipids as well as significantly increased plasma free fatty acids in rats. The increase in cholesterol was more prominent at the lower 200 ppm Pb dose. The impacts of these Pb-related changes in phospholipidosis and cholesterologenesis in the brain on downstream nervous system effects are not well characterized.

4.3.11 Lifestages and Time Periods of Pb Exposure and Neurodevelopmental Effects

Environmental exposures during critical lifestages can affect key physiological systems that orchestrate plasticity ([Feinberg, 2007](#)). Exposures during the prenatal and/or early postnatal period may be especially detrimental for neurodevelopmental effects because of active neuronal growth and/or synaptogenesis/pruning structure that occur during these periods ([Rice and Barone, 2000](#); [Nolte, 1993](#)). However, brain development has been shown to continue throughout adolescence. MRI studies of the Child Psychiatry Branch cohort of children and adults ages 3-30 years have shown peak total cerebral volume at age 10.5 and 14.5 years in females and males, respectively ([Giedd et al., 2009](#); [Lenroot and Giedd, 2006](#)). The volume of the cerebellum was found to peak two years later. Lateral ventricular volume and white and gray matter volume also were found to increase throughout adolescence. Gray matter volume peaked 1 to 3 years earlier in females than males. These observations that brain development is active throughout childhood and in adolescence indicate the potential for neurodevelopment to be altered later in childhood.

Epidemiologic studies consistently show that blood Pb levels measured during various lifestages and time periods, including the prenatal period, early childhood, later childhood, and averaged over multiple years, are associated with cognitive function decrements and increases in externalizing and internalizing behaviors. These observations of Pb-associated elevated risk of neurodevelopmental decrements in children are well supported by findings in animals that prenatal and/or early postnatal or lifetime Pb exposure alters brain development via changes in synaptic architecture ([Section 4.3.10.4](#)) and neuronal outgrowth ([Section 4.3.10.10](#)) and leads to impairments in memory and learning ([Section 4.3.2.3](#)) and increases in impulsivity ([Section 4.3.3.1](#)). In monkeys, Pb exposures during multiple lifestages and time periods, including lifetime, infancy, or juvenile to adulthood, resulted in impaired cognitive function, although not on all tests ([Rice, 1992b](#); [Rice and Gilbert, 1990a](#); [Rice, 1990](#); [Rice and Karpinski, 1988](#)). On one test of executive function in the same monkeys at ages 5-6 years, impairments were found with lifetime Pb exposure starting from birth or starting at age 400 days but not infancy-only exposure ([Rice and Gilbert, 1990b](#)). The observations indicate that

gestational or infancy Pb exposures are not necessary to induce cognitive function decrements in juvenile animals.

Unlike other organ systems, the unidirectional nature of CNS development limits the capability of the developing brain to compensate for cell loss, and environmentally-induced cell death can result in a permanent reduction in cell numbers ([Bayer, 1989](#)). Hence, when normal development is altered, the early effects have the potential to persist into adult life even in the absence of concurrent exposure, magnifying the potential public health impact. Some epidemiologic evidence indicates associations of earlier childhood blood or tooth Pb levels with cognitive function decrements, attention decrements, and increases in behaviors related to conduct disorders in adolescents or adults ([Mazumdar et al., 2011](#); [Fergusson et al., 2008](#); [Wright et al., 2008](#); [Ris et al., 2004](#); [Bellinger et al., 1994a](#); [Stiles and Bellinger, 1993](#)). These epidemiologic studies did not examine adult blood Pb levels, thus the relative influence of adult Pb exposure cannot be ascertained. In the Boston cohort, stronger associations observed for age 2 year blood Pb level than concurrent blood Pb level with FSIQ decrements at ages 57 months and 10 years indicated an effect of earlier rather than later childhood Pb exposures ([Bellinger et al., 1992](#); [1991](#)). The persistence of effects of early exposures is supported by findings of impaired learning in adult monkeys that had infancy-only Pb exposure ([Rice, 1992b](#); [Rice and Gilbert, 1990a](#); [Rice, 1990](#)). A few available toxicological studies also found that infancy Pb exposure (but not adult exposure in rats) led to neurodegenerative amyloid plaque formation in the brains of aged rodents and monkeys ([Section 4.3.9.1](#)).

With repeated assessments of children (prenatally to later childhood and early adulthood), the prospective cohort studies have provided data to compare the neurodevelopmental effects associated with blood Pb levels measured at different lifestages and time periods. In the collective body of evidence, cognitive function decrements in children have been associated with prenatal, early childhood, childhood average, and concurrent blood Pb levels, without clear indication of a single critical lifestage or duration of Pb exposure related to risk of neurodevelopmental effects in children. The identification of critical developmental periods with regard to risk of neurodevelopmental decrements from Pb exposure has been complicated by the high degree of correlation of the blood Pb levels of children over time and the confounding of age and peak blood Pb levels ([Hornung et al., 2009](#); [Lanphear et al., 2005](#); [Dietrich et al., 1993a](#)).

As described in detail in the 2006 Pb AQCD ([U.S. EPA, 2006b](#)), several studies with varying lengths of follow-up demonstrated associations of prenatal blood Pb levels (maternal and umbilical cord) with decrements in cognitive function throughout childhood and into early adulthood ([Section 4.3.2](#)). These findings are consistent with the observations of active CNS development occurring during prenatal development as

described above. Substantial fetal Pb exposure may occur from mobilization of maternal skeletal Pb stores, which may be related to past maternal Pb exposures ([Gulson et al., 2003](#); [Hu and Hernandez-Avila, 2002](#)), and the transfer of Pb across the placenta ([Section 3.2.2.4](#)). Among 94-211 mother-child pairs in Albany County, NY, maternal pregnancy-cord blood Pb level correlations of 0.53-0.81 were reported, depending on the stage of pregnancy, indicating the influence of maternal blood Pb levels on newborn blood Pb levels ([Schell et al., 2003](#)). Depending on the magnitude of child exposure, the contribution of maternal blood Pb on child blood Pb levels appears to diminish rapidly over a period of a few months following birth, after which child blood Pb levels may be influenced mainly by postnatal Pb exposures ([Section 3.4.1](#)). Thus, associations of neurodevelopmental outcomes assessed after infancy with postnatal blood Pb levels may reflect effects of postnatal Pb exposures.

In most studies of very young children, ages <2 years, decrements in MDI score were associated with higher prenatal (maternal or cord) and concurrent blood Pb levels ([Table 4-14](#)). Among studies that examined blood Pb levels at multiple time periods, some found a larger magnitude decrement in MDI per unit increase in prenatal blood Pb than concurrent blood Pb ([Hu et al., 2006](#); [Gomaa et al., 2002](#)) ([Table 4-14](#)). The mean was higher for prenatal blood Pb level than concurrent in one study ([Hu et al., 2006](#)), whereas another study reported similar means for prenatal and concurrent blood Pb levels ([Gomaa et al., 2002](#)). Bellinger et al. ([1987](#)) did not report effect estimates for concurrent blood Pb level and only indicated lack of statistically significant association with MDI. In the Yugoslavia cohort, per log increase in blood Pb level, the MDI decrement at age 2 years was larger for concurrent blood Pb than for prenatal cord blood Pb ([Wasserman et al., 1992](#)). Concurrent blood Pb levels were higher than prenatal cord blood Pb levels. The collective evidence indicates that both prenatal and postnatal child Pb exposures may contribute to neurodevelopmental effects in children at to age 2 years, with some indication that prenatal Pb exposure has a stronger effect.

Table 4-14 Associations of cognitive function with blood Pb levels measured at various lifestages and time periods in prospective studies.

Study ^a	Study Population and Methodological Details Within studies, effect estimates are presented in order of lifestage or time period of blood Pb measurement, with largest effect estimate in bold ^a	Blood Pb Metrics Analyzed (µg/dL)	Outcome Analyzed	Effect Estimate (95% CI) ^b
Cognitive Assessment at Age 2 Years and Younger				
Bellinger et al. (1987)	182 children followed from birth (1979-1981) to age 2 yr, Boston, MA area. Recruitment from birth hospital. High follow-up participation. Participants had higher cord blood Pb level, SES, HOME score, maternal education and IQ, lower maternal age, were white. Regression model adjusted for maternal age, education, race, IQ, years of smoking, and alcohol drinks/week in 3rd trimester, SES, HOME score, child sex, birth weight, gestational age, birth order.	Prenatal (cord) Mean (SD): 6.6 (3.2) Low: < 3 Medium: 6-7 High: ≥ 10	Overall Bayley MDI among Ages 6, 12, 18, and 24 mo	Score in high cord blood Pb group vs. Low cord blood: -4.8 (-7.3, -2.3)^c vs. Medium cord blood: -3.8 (-6.3, -1.3)^c Concurrent reported to not to be significantly associated with overall MDI, no quantitative data reported.
Dietrich et al. (1986)	245-280 children followed prenatally to age 6 mo, Cincinnati, OH No information on participation rate. Log linear regression model adjusted for birth weight, gestation, sex. Also considered potential confounding by SES, HOME score, prenatal smoking and alcohol use, maternal Fe binding capacity, child sex, race.	Mean (SD) Prenatal (maternal): 8.0 (3.8) Concurrent: 4.5 (2.9)	Bayley MDI Age 6 mo	Per log increase in blood Pb: Prenatal: -15 (-27, -3) Concurrent: -15 (-25, -5)
Hu et al. (2006)	83-146 children born 1997-1999 followed prenatally to age 2 yr, Mexico City, Mexico Recruitment from prenatal clinic. Moderate follow-up participation. Eligible similar to non-eligible. Log linear regression model adjusted for maternal age and IQ, child sex, current weight, height-for-age Z score, and concurrent blood Pb (in models examining prenatal blood Pb). Considered potential confounding by other unspecified factors.	Mean (SD) Prenatal (maternal 1st trimester): 7.1 (5.1) Prenatal avg NR Earlier childhood at 12 mo: 5.2 (3.4) Concurrent: 4.8 (3.7)	Bayley MDI Age 24 mo	Per log increase in blood Pb: Prenatal 1st trimester: -4.1 (-8.1, -0.17) Prenatal avg: -3.5 (-7.7, 0.63) Age 12 month: -2.4 (-6.2, 1.5) Concurrent: -1.0 (-3.9, 1.9)

Table 4-14 (Continued): Associations of cognitive function with blood Pb levels measured at various lifestages and time periods in prospective studies.

Study ^a	Study Population and Methodological Details Within studies, effect estimates are presented in order of lifestage or time period of blood Pb measurement, with largest effect estimate in bold ^a	Blood Pb Metrics Analyzed (µg/dL)	Outcome Analyzed	Effect Estimate (95% CI)^b
Gomaa et al. (2002)	161-197 children followed from birth to age 2 yr, Mexico City, Mexico Recruitment from birth hospital. Moderate participation but high retention, no selective attrition. Log linear regression model adjusted for maternal IQ, age, parents in home, breastfeeding duration, parental education, child hospitalization status, and sex. Did not consider potential confounding by parental caregiving quality.	Mean (SD) Prenatal (cord): 6.7 (3.4) Concurrent: 8.4 (4.6)	Bayley MDI Age 24 mo	Per log increase in blood Pb: Prenatal: -4.9 (-9.0, -0.89) Concurrent: -0.09 (-0.58, 0.40)
Wasserman et al. (1992)	392 children followed prenatally to age 2 yr, K. Mitrovica, Pristina, Yugoslavia. 53% live near Pb sources. High follow-up participation, no selective attrition. Log linear regression model adjusted for sex, birth order, birth weight, ethnic group, HOME score, maternal education, age, and IQ.	Mean (SD) Prenatal (cord): 14 (10) Concurrent: 35 K. Mitrovica, 8.5 Pristina	Bayley MDI Age 24 mo	Per log increase in blood Pb: Prenatal: -3.5 (-8.6, 1.7) Concurrent: -5.3 (-10, -0.53)
Ernhart et al. (1988; 1987)	146-165 children, followed prenatally to age 2 yr, Cleveland, OH Prospective. Recruitment at birth hospital. High follow-up participation. More white, higher IQ, nonalcohol using mothers not followed. 50% born to alcoholic mothers. Linear regression adjusted for age, race, sex, birth order, birth weight, parental education, maternal IQ, Authoritarian Family Ideology, HOME score.	Mean (SD) Prenatal cord: 6.0 (1.8) 6 mo: 10 (3.3) Concurrent: 17 (6.5)	Bayley MDI Age 2 yr	Variance estimates: Prenatal: 0.0003, t = -0.21 ^d Age 6 mo: 0.00, p = 0.95 ^d Concurrent: 0.00, p = 0.95 ^d
Cognitive Assessment at Age 3 Years and Older				
Canfield et al. (2003a)	171-172 children born 1994-1995 followed from age 6 mo to 5 yr, Rochester, NY Recruitment from study of dust control. 73% nonwhite. Moderate follow-up participation but no selective attrition. Linear regression model adjusted for child sex, Fe status, birth weight, maternal race, education, IQ, income, and prenatal smoking status, HOME score.	Mean (SD) Infancy avg (6-24 mo): 7.0 (3.8) Peak: 11 (7.1) Concurrent: 5.8 (4.1) Lifetime (to age 5 yr) avg: 7.4 (4.3)	FSIQ Stanford-Binet Age 5 yr	Per 1 µg/dL increase in blood Pb: Infancy avg: -0.53 (-0.93, -0.13) Peak: -0.26, (-0.47, -0.05) Concurrent: -0.61 (-0.99, -0.24) Lifetime avg: -0.57 (-0.93, -0.20)

Table 4-14 (Continued): Associations of cognitive function with blood Pb levels measured at various lifestages and time periods in prospective studies.

Study ^a	Study Population and Methodological Details Within studies, effect estimates are presented in order of lifestage or time period of blood Pb measurement, with largest effect estimate in bold ^a	Blood Pb Metrics Analyzed (µg/dL)	Outcome Analyzed	Effect Estimate (95% CI)^b
Wasserman et al. (1994)	332 children followed prenatally to ages 3-4 yr (1991), K. Mitrovica, Pristina, Yugoslavia Moderate follow-up participation. More participants were male, from K. Mitrovica, Albanian, and had lower maternal IQ and education and HOME score. Log linear regression model adjusted for HOME score, maternal age, IQ, and education, language, sibship size, birth weight, child sex.	Mean (SD) Prenatal (cord): 14 (11) Geometric means Concurrent K. Mitrovica: 40 Pristina: 9.6	FSIQ McCarthy GCI Age 4 yr	Per log increase in blood Pb: Prenatal cord: -7.1 (-12, -3.1) Age 2 yr: -10.4 (-15, -5.7) Concurrent: -9.4 (-14, -4.6)
Wasserman et al. (2000a)	390 children followed prenatally (1985-1996) to age 7 yr, K. Mitrovica, Pristina, Yugoslavia Moderate follow-up participation. More participants were from K. Mitrovica, Albanian, with lower maternal IQ and education and HOME score. Regression model adjusted for HOME score, maternal age, education, IQ, and # live births, child sex, birthweight, ethnicity, age.	Mean of average during period Prenatal:10 Age 0-2: 13 Age 2-3: 19 Age 2-4: 19 Age 2-5: 19 Age 2-7: 17	FSIQ McCarthy GCI, ages 3-4 yr WPPSI, age 5 yr WISC-III, age 7 yr	Prenatal: -6.1 (-8.7, -3.4) per log increase in blood Pb Postnatal blood Pb analyzed as categorical variable No increase prenatal to postnatal: Reference ≥50% increase in postnatal only from birth to age 2 yr: -1.8 (-3.5, -0.05) ^c ≥50% increase in postnatal from birth to age 2 yr and from age 2 yr onward: -2.7 (-4.9, -0.52) ^c
Bellinger et al. (1992)	148 children followed from birth (1979-1981) to age 10 yr, Boston, MA area Moderate follow-up participation. Participants had higher SES and HOME scores. Linear regression model adjusted for HOME score (age 10 and 5 yr), maternal race, age, IQ, and marital status, SES, child sex, birth order, and stress. Plus: # residence changes (2 yr), family stress, birth weight, # daycare situations to 5 yr (10 yr). Also considered potential confounding by psychiatric factors, child serum ferritin levels.	Mean (SD) Age 6 mo: 6.7 (7.0) Earlier childhood Age 2 yr: 6.5 (4.9) Concurrent: 2.9 (2.4)	FSIQ WISC-Revised Age 10 yr	Per 1 µg/dL increase in blood Pb: Age 6 mo: -0.13 (-0.42, 0.16) Age 2 yr: -0.58 (-0.99, -0.17) Concurrent -0.46 (-1.5, 0.56)

Table 4-14 (Continued): Associations of cognitive function with blood Pb levels measured at various lifestages and time periods in prospective studies.

Study ^a	Study Population and Methodological Details Within studies, effect estimates are presented in order of lifestage or time period of blood Pb measurement, with largest effect estimate in bold ^a	Blood Pb Metrics Analyzed (µg/dL)	Outcome Analyzed	Effect Estimate (95% CI)^b
Dietrich et al. (1993b)	199-231 children followed from birth (1979-1985) to age 6.5 yr, Cincinnati, OH Recruitment at prenatal clinic. High follow-up participation. Participants had slightly higher age 1 yr blood Pb levels. Primarily African-American. Linear regression model adjusted for HOME score, child birth weight, birth length, and sex, maternal IQ and prenatal smoking. Also considered potential confounding by perinatal complications, prenatal maternal substance abuse, nutritional status.	Mean (SD) Prenatal (maternal) 8.3 (3.7) Age 4-5 yr avg 11.8 (6.3) Concurrent: NR Lifetime (to age 6.5 yr) avg: NR	FSIQ WISC-Revised Age 6.5 yr	Per 1 µg/dL increase in blood Pb: Prenatal: 0.15 (-0.26, 0.56) Concurrent: -0.33 (-0.60, -0.06) Lifetime avg: -0.13 (-0.35, 0.09)
Baghurst et al. (1992)	494 children followed from birth (1979-1982) to ages 7-8 yr, Port Pirie, Australia Moderate follow-up participation. Participants had higher SES, more breastfeeding, less maternal smoking. Log linear regression model adjusted for sex, birth weight, birth order, feeding method, breastfeeding duration, parental education and smoking status, maternal age and IQ, SES, HOME score, parents living together.	Means of 2nd quartile Prenatal (cord): 7.4 Age 0-2 yr: 17 Lifetime (to age 7 yr) avg: 16	FSIQ WISC-Revised Age 7-8 yr	Per log increase in blood Pb: Prenatal: 0.6 (-2.1, 3.3) Age 0-2 yr: -4.6 (-8.7, -0.48) Lifetime avg: -3.7 (-8.6, 1.2)
Schnaas et al. (2006)	136-150 children followed from birth (1987-1992) to age 10 yr, Mexico City, Mexico. Recruitment at prenatal clinic. Low follow-up participation. Participants had higher SES, FSIQ, higher blood Pb level before age 5 yr, lower at older ages. Log linear mixed effects model adjusted for SES, maternal IQ, child sex, birth weight, & postnatal blood Pb, age of 1st FSIQ test, random slope for subject. Also considered potential confounding by HOME score. Covariates assessed in pregnancy or within age 6 mo.	Geometric Mean (5th-95th) Prenatal (maternal 28-36 week): 7.8 (2.5-25) Age 5 yr: 9.3 (3.8-18) Age 6-10 yr avg: 6.2 (range = 2.2-19)	FSIQ WISC-Revised Ages 6-10 yr	Per log increase in blood Pb: Prenatal: -4.0 (-6.4, -1.7) Age 5 yr: -0.32 (-4.3, 3.4) Age 6-10 yr avg: -2.5 (-4.1, -0.81)

Table 4-14 (Continued): Associations of cognitive function with blood Pb levels measured at various lifestages and time periods in prospective studies.

Study ^a	Study Population and Methodological Details Within studies, effect estimates are presented in order of lifestage or time period of blood Pb measurement, with largest effect estimate in bold ^a	Blood Pb Metrics Analyzed (µg/dL)	Outcome Analyzed	Effect Estimate (95% CI)^b
Ris et al. (2004)	195 children followed prenatally (1979-1985) to age 15-17 yr, Cincinnati, OH Prospective. Recruitment at prenatal clinic. High follow-up participation, no selective attrition. Mostly African-American. Linear regression model adjusted for SES, maternal IQ, HOME, adolescent marijuana use, obstetrical complications. Also considered potential confounding by birth outcomes, maternal age, maternal prenatal smoking, alcohol, marijuana, and narcotics use, # previous abortions, stillbirths, gravidity, parity, public assistance, child sex, age, health and Fe status, caregiver education.	Prenatal, Earlier childhood (age 6.5 yr), Earlier childhood avg (age 3-78 mo): NR	Learning/IQ factor score WISC-III, WRAT-3 indices Ages 15-17 yr	Per 1 µg/dL increase in blood Pb: Prenatal: -0.08 (-0.18, 0.03) Age 6.5 yr: -0.08 (-0.17, 0.00) Age 3-78 mo avg: -0.03 (-0.09, 0.02)
Lanphear et al. (2005)	1,333 children pooled from Boston, Cincinnati, Cleveland, Mexico City, Port Pirie, Rochester, and Yugoslavia cohorts. Uniform analytic method applied to cohorts from diverse locations and demographic characteristics. Blood Pb levels and FSIQ measured at different ages. Several sensitivity analyses to examine heterogeneity of results by cohort, confounding, and model specification. Log linear regression model adjusted for HOME score, birth weight, maternal IQ and education. Also considered potential confounding by child sex, birth order, marital status, maternal age, prenatal smoking status and alcohol use.	Median (5th-95th) Early childhood (mean ages 6-24 mo): 12.7 (4.0-35) Peak: 18 (6.2-47) Lifetime avg (to ages 4.8-10 yr): 12 (4.1-35) Concurrent: 9.7 (2.5-33)	FSIQ Various tests Ages 4.8-10 yr	Per log increase in blood Pb: Early childhood: -2.0 (-3.3, -0.81) Peak: -2.9 (-4.1, -1.6) Concurrent: -3.0 (-4.3, -1.8) Lifetime avg: -2.7 (-3.7, -1.7)

Table 4-14 (Continued): Associations of cognitive function with blood Pb levels measured at various lifestages and time periods in prospective studies.

Study ^a	Study Population and Methodological Details		Outcome Analyzed	Effect Estimate (95% CI) ^b
	Within studies, effect estimates are presented in order of lifestage or time period of blood Pb measurement, with largest effect estimate in bold ^a	Blood Pb Metrics Analyzed (µg/dL)		
Pocock et al. (1994)	Meta-analysis of 5 prospective studies (1,260 children) from Port Pirie and Sydney, Australia, Cincinnati, Cleveland, Boston Meta-analysis of combining covariate-adjusted effect estimates from individual studies.	Earlier childhood (2 yr) range in means: 6.8-21 Around birth, Postnatal: NR	FSIQ Various tests Ages 5-10 yr	Per doubling of blood Pb from 10-20 µg/dL: Around birth: 0.18 (-1.0, 1.4) Age 2 yr: -1.9 (-2.8, -0.85) Postnatal mean: -0.88 (-2.0, 0.26)

MDI = Mental Development Index, FSIQ = full-scale IQ, NR = Not reported, McCarthy GCI = McCarthy General Cognitive Index, WPPSI = Wechsler Preschool and Primary Scale of Intelligence, WISC = Wechsler Intelligence Scale for Children

^aResults are presented first for MDI in children up to age 2 years, then for FSIQ in children ages 3 years and older. Within studies, effect estimates are presented in order of increasing lifestage or time period of blood Pb measurement, with the largest effect estimate in bold. After age of assessment, studies are grouped by strength of study design, representativeness of the study population characteristics and blood Pb levels examined, and extent of consideration for potential confounding. There is not necessarily a continuum of decreasing strength across studies.

^bEffect estimates are standardized to a 1 µg/dL or log increase in blood Pb level in analyses of blood Pb as a continuous variable.

^cEffect estimates represent comparisons between children in different categories of blood Pb level, with children in the lower blood Pb category serving as the reference group.

^dSufficient data were not available to calculate 95% CI.

Prenatal and early postnatal (age 6 month) blood Pb levels also were associated with cognitive function in studies that included school-aged children (ages 5-17 years) ([Table 4-14](#)). However, most of these studies also found cognitive function decrements in association with postnatal blood Pb levels, and results did not identify an individual critical postnatal time period of blood Pb measurement associated with cognitive function decrements. In the Yugoslavia cohort, the FSIQ decrement per unit increase in blood Pb level was larger for prenatal maternal than concurrent blood Pb when children were examined at ages 3-7 years ([Wasserman et al., 2000a](#)), but larger for concurrent than prenatal maternal or cord blood Pb when children were examined at age 4 years ([Wasserman et al., 1994](#)). In the Cincinnati cohort, the association with FSIQ was stronger for concurrent than for prenatal blood Pb levels at age 6.5 years ([Dietrich et al., 1993b](#)), but in adolescents ages 15-17 years, increases in both prenatal and earlier childhood (age 6.5 years) blood Pb levels were associated with decrements in a learning/memory composite score ([Ris et al., 2004](#)). In the Boston and Port Pirie cohorts, increases in age 2 year or 0-2 year average blood Pb levels were associated with larger FSIQ decrements at ages 10 and 7 years than were concurrent or lifetime average blood Pb levels, respectively ([Baghurst et al., 1992](#); [Bellinger et al., 1992](#)). However, in the Port Pirie cohort, there was an association found for lifetime average (to age 7 years) blood Pb level. Among children ages 6-10 years in Mexico City, per unit increase, prenatal blood Pb level was associated with a larger FSIQ decrement than were blood Pb levels measured at each age from 1 to 5 years or averaged from age 6 to 10 years ([Schnaas et al., 2006](#)). In contrast, results from the Rochester cohort at age 5 years indicated that the FSIQ decrement per unit increase in blood Pb level was larger for lifetime average and concurrent blood Pb than peak blood Pb ([Canfield et al., 2003a](#)). Collectively, the epidemiologic findings indicate that blood Pb levels measured at various postnatal time periods, early childhood (e.g., age 2 year), lifetime average (e.g., to 7 years), later childhood (e.g., age 6-10 year average), and concurrent blood Pb levels are associated with decrements in cognitive function when assessed in populations that include school-aged children ages 5-17 years.

Consistent with individual studies, analyses combining studies pointed to associations of FSIQ in school-aged children with blood Pb levels measured at various lifestages and time periods. The analysis pooling data from seven prospective studies found that increases in early childhood (age 6-24 month average), peak, concurrent, and lifetime average blood Pb levels were associated with decreases in FSIQ in children ages 4.8-10 years ([Table 4-14](#)). Investigators reported that the model with concurrent blood Pb level explained the largest proportion of variance in FSIQ (R^2) ([Lanphear et al., 2005](#)). In a meta-analysis of results from five prospective studies ([Pocock et al., 1994](#)), the decrease in FSIQ per unit increase in blood Pb level was larger for peak (around age 2 years) blood Pb level than blood Pb level measured around birth or after age 2 years. Deciduous

tooth Pb levels have been associated with decrements in cognitive function, decrements in attention, as well as increases in impulsivity and hyperactivity in children and young adults ([Table 4-8](#), [Table 4-9](#), and [Table 4-11](#)). These results that indicate cumulative Pb exposure over several years may contribute to neurodevelopmental effects in children. In school-aged children, concurrent blood Pb levels reflect recent exposures and past Pb exposures that are mobilized from bone remodeling to blood. Thus, associations with concurrent blood Pb levels also may reflect an effect of cumulative past and more recent Pb exposures.

Studies conducted in the Cincinnati cohort examined diverse nervous system effects and found that prenatal or neonatal blood Pb levels were associated with poorer auditory processing in children ages 5 years ([Dietrich et al., 1992](#)) and higher parental ratings of delinquent behavior in adolescents ages 15-17 years ([Dietrich et al., 2001](#)) but inconsistently with motor function decrements at ages 4-10 years ([Bhattacharya et al., 2006](#); [Bhattacharya et al., 1995](#); [Dietrich et al., 1993a](#)) and not with cognitive function in children at age 6 years ([Dietrich et al., 1993b](#)). These findings suggest that the critical timing of Pb exposure may vary among nervous system endpoints.

Some studies have aimed to improve the characterization of important lifestages and time periods of Pb exposure by examining children in whom blood Pb levels are not strongly correlated over time (i.e., children whose blood Pb level ranking changed over time) ([Hornung et al., 2009](#); [Schnaas et al., 2006](#); [Chen et al., 2005a](#); [Wasserman et al., 2000a](#); [Tong et al., 1998](#); [Bellinger et al., 1990](#)). Some calculated specific blood Pb metrics that reflected the change over time (i.e., ratio of blood Pb measured at different times, amount of change over time), and collectively most results indicated FSIQ decrements in association with higher concurrent blood Pb levels but did not conclusively demonstrate stronger findings for early or concurrent blood Pb levels ([Table 4-15](#)).

Schnaas et al. ([2006](#)) did not calculate a blood Pb level metric to reflect the change over time but found a weak correlation between maternal blood Pb levels at 28-36 weeks of pregnancy and repeated measures of child blood Pb level between ages 1 and 10 years (Pearson $r \leq 0.23$). In this study, children in Mexico City were followed prenatally to age 10 years. In models analyzing blood Pb levels at different ages individually or together in one mixed effects model that included prenatal and multiple postnatal blood Pb measures, the decrement in FSIQ per unit increase in blood Pb level was largest for maternal 28-36 week blood Pb level ([Table 4-14](#)). In the model that included blood Pb level at multiple ages, analysis of variance inflation factors indicated a lack of collinearity among the serial blood Pb measures

Table 4-15 Comparisons of blood Pb-cognitive function associations in groups of children with different temporal trends in blood Pb levels.

Study	Study Population and Methodological Details ^a	Blood Pb Analyzed (µg/dL)	Outcome Analyzed	Effect Estimate (95% CI)
Hornung et al. (2009)	397 children (born 1979-1995) followed from age 2 to 6 yr, Rochester, NY and Cincinnati, OH. Moderate follow-up participation, representative of full cohorts. Regression model adjusted for city, HOME score, birth weight, maternal IQ and education, log childhood average blood Pb level.	Geometric mean (5th-95th): Early childhood age 2 yr: 8.9 (3.0-24) Concurrent: 6.0 (1.9-18) 6.3% with higher age 6 yr than age 2 yr blood Pb level	FSIQ WPPSI and WISC-R Age 6 yr	Difference in FSIQ at age 6 yr per 1 unit increase in age 6:2 year blood Pb ratio: -7.0 (-10, -4.0) Estimates a 7-point lower FSIQ in children with 50% higher blood Pb level at age 6 than 2 years (ratio = 1.5) compared to children with a 50% lower blood Pb level at age 6 than 2 years (ratio = 0.5)
Bellinger et al. (1990)	170 children followed prenatally to age 57 mo, Boston, MA area High follow-up participation, no comparison with nonparticipants. Log regression adjusted for HOME score, SES, maternal IQ and age, sex, ethnicity.	High prenatal (cord): >10 Concurrent Low: <3 Medium: 3-10 High: >10	Change in z-score McCarthy GCI at age 57 mo MDI at 24 mo	High prenatal/Low concurrent 0.42 (-0.15, 0.99) High prenatal/Medium concurrent 0.15 (-0.14, 0.44) High prenatal/High concurrent -0.15 (-0.56, 0.26)
Wasserman et al. (2000a)	390 children followed prenatally (1985-1986) to age 7 yr, K. Mitrovica, Pristina, Yugoslavia Moderate follow-up participation. More participants were from K. Mitrovica, Albanian, with lower maternal IQ and education and HOME score. Regression model adjusted for HOME score, maternal age, education, IQ, and # live births, child sex, birthweight, ethnicity, age.	Mean of average during period Prenatal:10 Age 0-2: 13 Age 2-3: 19 Age 2-4: 19 Age 2-5: 19 Age 2-7: 17	FSIQ McCarthy GCI, ages 3-4 yr WPPSI, age 5 yr WISC-III, age 7 yr	No change from prenatal to postnatal: Reference ≥50% increase prenatal to postnatal only birth to age 2 yr: -1.8 (-3.5, -0.05) ≥50% increase prenatal to postnatal from birth to age 2 yr and from age 2 yr onward: -2.7 (-4.9, -0.52)

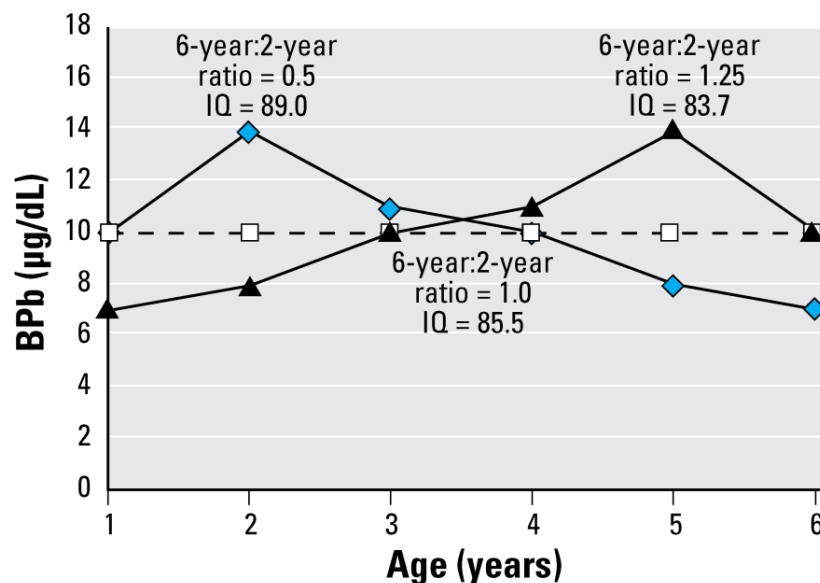
Table 4-15 (Continued): Comparisons of blood Pb-cognitive function associations in groups of children with different temporal trends in blood Pb levels.

Study	Study Population and Methodological Details ^a	Blood Pb Analyzed (µg/dL)	Outcome Analyzed	Effect Estimate (95% CI)
Tong et al. (1998)	326 children followed from birth (1979-1982) to age 11-13 yr, Port Pirie, Australia Moderate follow-up participation. Participants had weaker early blood Pb-cognitive function association. ANOVA to compare mean change in FSIQ among children with different degrees of change in blood Pb across time.	Means Early childhood age 2 yr: 21 Age 11-13 yr: 7.9	Mean change in cognitive function z-scores between ages 2 and 11-13 yr Bayley MDI, age 2 yr WISC-R, ages 11-13 yr	<10.2 µg/dL decline: 0.03 (-0.15, 0.21) 10.2-16.2 µg/dL decline: 0.04 (-0.15, 0.23) >16.2 µg/dL decline: -0.01 (-0.20, 0.18) p = 0.74 for difference among groups
Chen et al. (2005a)	617 children participating in the TLC trial from age 12-33 mo to age 7 yr, Baltimore, MD; Cincinnati, OH; Newark, NJ 77% African-American. 50% given chelation at ages 12-33 mo, blood Pb levels 20-44 µg/dL. High follow-up participation. Regression model adjusted for city, race, sex, age at blood Pb measurement, language, single parent, caregiver IQ, parental education and employment. Considered potential confounding by chelation but not parental caregiving quality.	Mean (SD): Age 2 yr: 26 (5.1) Age 5 yr: 12 (5.2) Age 7 yr: 8.0 (4.0) Low age 2 yr: <24.9 Low age 7 yr: <7.2	WISC-III Age 7 yr	Difference in age 7 FSIQ Low age 2, Low age 7: Reference Low age 2, High age 7: -3.6 (-6.4, -0.7) High age 2, Low age 7: 0 (-2.8, 2.7) High age 2, High age 7: -3.7 (-6.2, -1.3)

WPPSI = Wechsler Preschool and Primary Scale of Intelligence, WISC = Wechsler Intelligence Scale for Children, GCI = General Cognitive Index

^aStudies are grouped by strength of study design, representativeness of the study population characteristics and blood Pb levels examined, and extent of consideration for potential confounding. There is not necessarily a continuum of decreasing strength across studies.

Combining the Cincinnati and Rochester cohorts ($n = 397$), Hornung et al. (2009) analyzed the ratio of age 6 blood Pb level to age 2 year blood Pb level to assess the association of FSIQ at age 6 years with a change in blood Pb level over time. Blood Pb levels measured at various ages were highly correlated ($r = 0.64$ - 0.96), but investigators found a range of temporal trends in blood Pb levels (range in age 6:2 year blood Pb level: 0.11 - 4.1 , mean: 0.58). This range was analyzed in the model as the predictor. The model estimated that for children with the same average (ages 6 months-6 years) blood Pb level, those with a higher age 6:2 year blood Pb ratio had a lower FSIQ at age 6 years (Table 4-15). Although 6.3% of children had a ratio > 1 , representing higher age 6 than age 2 blood Pb level, model results were not dependent on these data points. An example of model results is illustrated in Figure 4-14. The three temporal blood Pb patterns have similar areas under the curve, indicating similar childhood average blood Pb levels. Thus, differences in FSIQ more likely would be attributable to differences in temporal exposure pattern. Among the three blood Pb patterns illustrated, the model estimated the lowest FSIQ for children with an age 6:2 year blood Pb ratio of 1.25 or children who had a 25% increase in blood Pb level from 2 to 6 years of age (Figure 4-14).



Note: FSIQ was estimated for different patterns of blood Pb level changes over time: 1) peak at age 2 years, i.e., age 6:2 year ratio = 0.5 (blue diamonds); 2) peak at 5 years, i.e., age 6:2 year ratio = 1.25 (black triangles); and 3) constant blood Pb level, i.e., age 6:2 year ratio = 1 (white squares). The 3 patterns have a similar cumulative blood Pb level ($10 \mu\text{g/dL}$) as indicated the areas under the curve. Among the 3 patterns, the lowest FSIQ at age 6 years was estimated for blood Pb level peaking at age 5 years.

Source: Hornung et al. (2009)

Figure 4-14 Estimated FSIQ for three different temporal patterns in blood Pb level from ages 2 to 6 years in Rochester and Cincinnati cohorts.

Results from both the Boston and Yugoslavia cohorts demonstrated cognitive function decrements in children with increases in blood Pb level from the prenatal to postnatal period ([Wasserman et al., 2000a](#); [Bellinger et al., 1990](#)) ([Table 4-15](#)). In the Boston cohort, children with high prenatal cord (≥ 10 $\mu\text{g/dL}$) and high concurrent (≥ 10 $\mu\text{g/dL}$) blood Pb level had a decrease in cognitive function from age 24 to 57 months ([Bellinger et al., 1990](#)). In contrast, children with high prenatal blood Pb but low concurrent (<3 $\mu\text{g/dL}$) blood Pb level had an increase in cognitive function from age 24 to 57 months ([Table 4-15](#)). Findings from this study indicated that by age 5 years, children with higher prenatal blood Pb levels appeared to recover the Pb-associated decrements in cognitive function unless concurrent blood Pb levels remained high. The investigators also demonstrated that optimal sociodemographic characteristics (e.g., higher HOME score, SES, maternal IQ and age, female) also protected against decrements in cognitive function associated with higher postnatal blood Pb levels. Similarly, environmental enrichment with items such as toys, tunnels, and running wheels in cages given after gestational-lactational Pb exposure was found to protect rats from deficits in spatial learning in adulthood ([Guilarte et al., 2003](#)). The Yugoslavia study examined in detail during what periods of postnatal development were increases in blood Pb level associated with FSIQ decrements. Children with at least a 50% increase in blood Pb level from the prenatal period only up to age 2 year (early postnatal increase) and children with increases to age 2 years and to 7 years (early and later postnatal increase) had lower FSIQ compared with children with no increase in blood Pb level from the prenatal or postnatal period ([Wasserman et al., 2000a](#)) ([Table 4-15](#)). The decrease in FSIQ was greater among children with both early and later postnatal increases in blood Pb level. Collectively, these results from the Boston and Yugoslavia cohorts suggest that cognitive development is not fixed early in childhood and can be affected negatively or positively by postnatal Pb exposures or other factors that influence cognitive function.

Chen et al. ([2005a](#)) found a stronger influence of higher concurrent than age 2 year blood Pb levels on FSIQ at age 7 years among children participating in a multi-city chelation trial. Children with higher concurrent blood Pb levels ($\geq$ median 7.2 $\mu\text{g/dL}$) had lower FSIQ at age 7 years, regardless of whether blood Pb level at age 2 years was low or high (less than or greater than the median of 24.9 $\mu\text{g/dL}$, respectively). Blood Pb levels at ages 2 and 7 years were weakly correlated ($r = 0.27$). In another analysis of the same study population ([Liu et al., 2002](#)) plus analysis of another chelation trial, results directly demonstrated increases in cognitive function in children with decreases in blood Pb levels over follow-up of 6 to 36 months ([Ruff et al., 1993](#)). Liu et al. ([2002](#)) found such an association in the group not receiving chelation. Because these children had initial high blood Pb levels (20-55 $\mu\text{g/dL}$) at ages 1 to 7 years, the findings may have limited generalizability to the general population of children currently living in the U.S.

Examining an older cohort (Port Pirie, Australia) at ages 11-13 years, Tong et al. ([1998](#)) found some evidence of persistence of effects of higher early-life blood Pb level ([Table 4-15](#)). This conclusion was based on the analysis of groups of children with different degrees of decline in blood Pb levels between ages 2 and 11-13 years. Although the mean blood Pb level in the study population declined overall from 21.2 µg/dL at age 2 years to 7.9 µg/dL at age 11-13 years, the magnitude of decline varied among children. The change in cognitive function between ages 2 and 11-13 years did not significantly differ between children with the largest decline (>16 µg/dL) in blood Pb level over that time period and children with the smaller decline (<10 µg/dL) ([Table 4-15](#)). Children with the largest decline in blood Pb level between ages 7 and 11-13 years had a slightly smaller decline in FSIQ over that time period. Thus, the findings indicated that cognitive function assessed in adolescents may be influenced by higher early-life blood Pb levels but also suggest some impact of more recent declines in blood Pb level. The results do not preclude an independent association with concurrent blood Pb level.

To conclude, the collective body of epidemiologic evidence does not strongly identify an individual critical lifestage or timing of Pb exposure with regard to neurodevelopmental effects in children. Cognitive function decrements and increases in externalizing and internalizing behaviors assessed at ages 3-24 years have been associated with prenatal, early childhood (e.g., age 1 or 2 year), lifetime average (to age 4-5, 7, or 11-13 years), and concurrent (at ages 3-17 years) blood Pb levels as well as with childhood tooth Pb levels. The identification of critical lifestages and time periods of Pb exposure is complicated further by the fact that blood Pb levels in older children, although affected by recent exposure, are also influenced by Pb stored in bone due to rapid growth-related bone turnover in children relative to adults. Thus, associations of neurodevelopmental effects with concurrent blood Pb level in children may reflect the effects of past and/or recent Pb exposures ([Section 3.3.5.1](#)). Evidence indicates that prenatal blood Pb levels are associated with mental development in very young children <age 2 years. However, in several studies, increases in postnatal (earlier childhood, lifetime average, concurrent) blood Pb levels were associated with larger cognitive function decrements in children ages 4-10 years than were increases in prenatal blood Pb levels ([Table 4-14](#)). These results suggest that per unit increase, postnatal Pb exposures that are reflected in concurrent or cumulative blood Pb levels or tooth Pb levels may have a larger magnitude of effect on cognitive function decrements as children age. Results from some studies indicated higher cognitive function in children at ages 1-8 years who had declines in blood Pb level over durations of 6 months to 5 years compared with children with smaller declines, no change, or increases in blood Pb levels ([Hornung et al., 2009](#); [Chen et al., 2005a](#); [Liu et al., 2002](#); [Ruff et al., 1993](#); [Bellinger et al., 1990](#)), providing some evidence that the effects of early Pb exposure on cognitive function are not fixed. These observations were made with declines from higher blood Pb levels at baseline (20-55

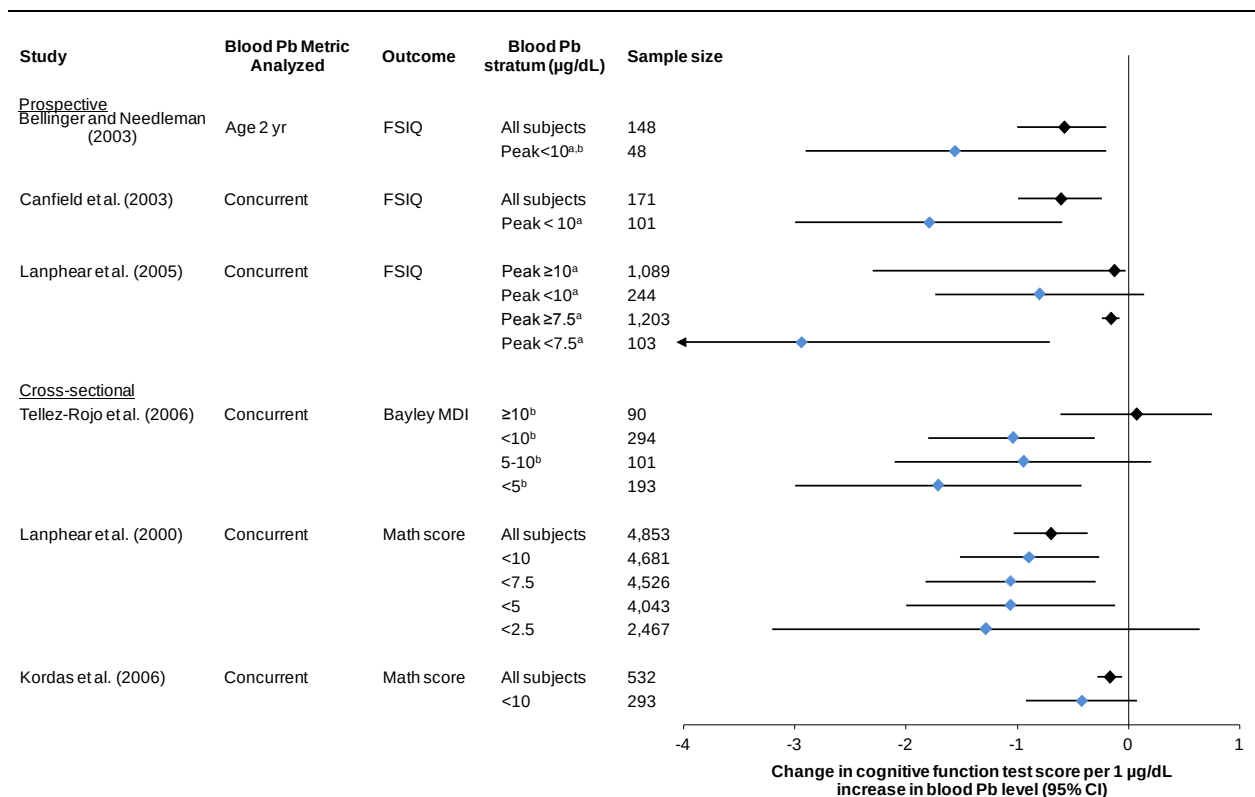
µg/dL) or larger declines over time (i.e., 8, 14 µg/dL) than those expected for most of the current population of U.S. children. Nonetheless, these findings are consistent with the understanding that the nervous system continues to develop throughout childhood. The epidemiologic evidence for associations of neurodevelopmental effects with multiple lifestages or time periods of Pb exposure, including more recent exposures, is supported by evidence in monkeys for Pb exposures during multiple lifestages and time periods, including infancy only, lifetime starting from birth, or lifetime starting during the juvenile period, inducing impairments in cognitive function when assessed between ages 6 and 10 years ([Rice, 1992b](#); [Rice and Gilbert, 1990a](#); [Rice, 1990](#); [Rice and Gilbert, 1990b](#); [Rice and Karpinski, 1988](#)).

4.3.12 Examination of the Pb Concentration-Response Relationship

With successive Pb AQCD and supplements, epidemiologic and toxicological studies find that progressively lower blood Pb levels are associated with effects on cognitive function and behavior. For example, among children, such effects were observed in association with blood Pb levels in the range of 10-15 µg/dL (for prenatal maternal or cord) in the 1986 Addendum ([U.S. EPA, 1986c](#)) and 1990 Supplement to the 1986 Addendum ([U.S. EPA, 1990a](#)) and 10 µg/dL and lower in the 2006 Pb AQCD ([U.S. EPA, 2006b](#)). Further, in the 2006 Pb AQCD, several individual studies and pooled analyses estimated a supralinear concentration-response relationship in children, i.e., greater decrements in cognitive function per unit increase in blood Pb level among children in lower strata of blood Pb levels compared with children in higher strata of blood Pb level ([Figure 4-15](#) and [Table 4-16](#)). Some evidence in animals also indicates nonlinear concentration-response relationships for various endpoints, including those related to learning impairments. Some toxicological evidence points to larger absolute effects in lower Pb exposure groups (relative to control groups) than in higher exposure groups. However, these toxicological findings of U- or inverted U-shaped relationships are distinct from epidemiologic findings of supralinear relationships in that some toxicological relationships do not indicate Pb-induced impairments at higher exposure concentrations.

Several prospective epidemiologic studies found supralinear concentration-response relationships for concurrent, early childhood (age 2 year), and lifetime average (to age 5 years) blood Pb levels and cognitive function decrements in children ages 2-16 years. Most studies used a blood Pb level of 10 µg/dL to define lower and higher strata and reported mean or median blood Pb levels in the lower strata of 3-7 µg/dL ([Bellinger, 2008](#); [Canfield, 2008](#); [Hornung, 2008](#); [Téllez-Rojo, 2008](#)). Except for the pooled analysis, the lower strata of blood Pb levels comprised >30% of the study population,

indicating that the blood Pb-cognitive function relationships calculated for the lower strata likely are not outliers or unrepresentative of the overall study population. Studies in the Boston and Rochester cohorts each examined different ages of blood Pb and FSIQ but lower blood Pb levels compared to other studies (means ~6 µg/dL), and a greater FSIQ decrement per unit increase in concurrent blood Pb level was found for children whose peak blood Pb levels < 10 µg/dL compared with all children examined ([Bellinger and Needleman, 2003](#); [Canfield et al., 2003a](#); [Bellinger et al., 1992](#)). A nonlinear blood Pb-FSIQ relationship also was indicated in pooled analyses of seven prospective studies involving a wider range of blood Pb levels (5th-95th percentiles 2.5-33.2 µg/dL) by statistical significance of blood Pb level modeled as quadratic and cubic functions ([Lanphear et al., 2005](#)) or better fit for a log-linear than linear model ([Rothenberg and Rothenberg, 2005](#)).



^aStrata were defined by the peak blood Pb level measured in child at any point during follow up. ^b95% CI estimated from reported p-value.

Note: Results are presented first for prospective analyses then for cross-sectional analyses. FSIQ = full-scale IQ, MDI = mental development index. Effect estimates (concentration-response) are presented for a 1 µg/dL increase in blood Pb level. Black symbols represent effect estimates among all subjects or subjects in the higher blood Pb stratum. Blue symbols represent effect estimates in lower blood Pb strata.

Figure 4-15 Comparison of associations between blood Pb level and cognitive function among various blood Pb strata.

Table 4-16 Additional characteristics and quantitative results for studies presented in Figure 4-15.

Study	Study Population and Methodological Details ^a	Blood Pb Metric Analyzed (µg/dL)	Outcome	Blood Pb stratum (µg/dL)	Effect Estimate (95% CI) ^b
Bellinger et al. (1992) Bellinger and Needleman (2003) Bellinger (2008) U.S. EPA (2006b)	148 children followed from birth (1979-1981) to age 10 yr, Boston area, MA. Prospective. Recruitment at birth hospital. Participation by 59% of original cohort but 88% from age 5 yr. Participants had higher SES, HOME score. Linear regression model adjusted for HOME score (age 10 and 5), child stress events, race, maternal IQ, age, marital status, SES, sex, birth order, # residence changes before age 5 yr	Early childhood (age 2 yr) Mean (SD) All subjects: 6.5 (4.9) Peak <10: 3.8 (range: 1-9.3) Detection limit not reported	FSIQ WISC-R Age 10 yr	All 148 subjects 48 subjects peak <10	-0.58 (-1.0, -0.17) -1.6 (-2.9, -0.2)
Canfield et al. (2003a) Canfield (2008)	171 children born 1994-1995 followed from age 6 mo to 5 yr, Rochester, NY. Prospective. Recruitment from study of dust control. 73% nonwhite. Moderate follow-up participation but no selective attrition. Mixed effects models adjusted for child sex, Fe status, and birth weight, maternal race, education, IQ, income and prenatal smoking, HOME score	Concurrent Mean (SD) All subjects: 5.8 (4.1) Peak <10: 3.3 (range: 0.5-8.4) Detection limit = 1	FSIQ Stanford-Binet Age 5 yr	All 171 subjects 101 subjects peak <10	-0.61 (-0.99, -0.24) -1.8 (-3.0, -0.60)
Jusko et al. (2008)	174 children born 1994-1995 followed from age 6 mo to 6 yr, Rochester, NY. Prospective. Same cohort as above. High follow-up participation. Higher follow-up of nonwhite, higher peak blood Pb, lower maternal IQ. Generalized additive model with LOESS adjusted for sex, birth weight, transferrin saturation, maternal race, IQ, education, and prenatal smoking status, HOME score (6 yr), family income.	Peak (6 mo-6 yr) Mean (SD): 11.4 (7.3)	FSIQ WPPSI-R Age 6 yr	Peak 20-30, n not given Peak 10-20, n not given 96 subjects, peak 2.1-10	-0.15 ^c -0.32 ^c -1.2 ^c

Table 4-16 (Continued): Additional characteristics and quantitative results for studies presented in Figure 4-15.

Study	Study Population and Methodological Details ^a	Blood Pb Metric Analyzed (µg/dL)	Outcome	Blood Pb stratum (µg/dL)	Effect Estimate (95% CI) ^b
Lanphear et al. (2005) Hornung (2008)	1,333 children pooled from Boston, Cincinnati, Cleveland, Mexico City, Port Pirie, Rochester, and Yugoslavia cohorts. Prospective. Large, uniform analysis pooling diverse cohorts. Included 84% of eligible children. Linear regression model adjusted for HOME score, birth weight, maternal IQ and education. Considered child sex, marital status, birth order, prenatal alcohol consumption and smoking status, maternal age.	Concurrent Mean (range) Peak ≥ 10: 13.9 (0.1-71.7) Peak <10: 4.3 (0.1-9.8) Peak ≥ 7.5: 12.9 (0.1-71.7) Peak <7.5: 3.2 (0.9-7.4)	FSIQ Various tests Ages 4.8-10 yr	1,089 subjects peak ≥ 10 244 subjects peak <10 1,203 subjects peak ≥ 7.5 103 subjects peak <7.5	-0.13 (-2.3, -0.03) -0.80 (-1.7, 0.14) -0.16 (-0.24, -0.08) -2.9 (-5.2, -0.71)
Tellez-Rojo et al. (2006) Tellez-Rojo (2008)	384 children followed from birth (1994-1995, 1997-1999) to age 2 yr, Mexico City, Mexico. Cross-sectional. Recruitment from prenatal clinic or birth hospital. Participants had higher maternal education, lower cord blood Pb. Linear regression model adjusted for sex, birth weight, maternal IQ. Considered maternal age and other unspecified factors.	Concurrent Mean (SD) ≥ 10: NR <10: 4.3 (2.3) 5-10: 6.9 (1.4) <5: 2.9 (1.1)	Bayley MDI Age 2 yr	90 subjects ≥ 10 294 subjects <10 101 subjects 5-10 193 subjects <5	0.07 (-0.61, 0.75) ^c -1.0 (-1.8, -0.25) ^c -0.94 (-2.1, 0.23) ^c -1.7 (-3.0, -0.42) ^c
Lanphear et al. (2000)	4,853 children ages 6-16 yr, NHANES III 1988-1994. Cross-sectional. Large study of multiple exposures and outcomes. Linear regression model adjusted for U.S. region, sex, race/ethnicity, reference adult education, poverty index ratio, marital status, serum ferritin level, serum cotinine level. Did not consider parental IQ or caregiving quality.	Concurrent Geometric mean (SD) All subjects: 1.9 (7.0) Subgroups: NR	Math Score WRAT Ages 6-16 yr	All 4,853 subjects 4,681 subjects <10 4,526 subjects <7.5 4,043 subjects <5 2,467 subjects <2.5	-0.70 (-1.0, -0.37) -0.89 (-1.5, -0.26) -1.1 (-1.8, -0.30) -1.1 (-2.0, -0.12) -1.3 (-3.2, 0.64)

Table 4-16 (Continued): Additional characteristics and quantitative results for studies presented in Figure 4-15.

Study	Study Population and Methodological Details ^a	Blood Pb Metric Analyzed (µg/dL)	Outcome	Blood Pb stratum (µg/dL)	Effect Estimate (95% CI) ^b
Kordas et al. (2006)	532 children ages 6-8 yr, Torreon, Mexico Cross-sectional. Recruitment at schools. Residence near metal foundry. High participation. Linear regression model adjusted for sex, age, hemoglobin, family possessions, forgetting homework, house ownership, crowding, maternal education, birth order, family structure, urine arsenic, tester, school. Did not consider parental IQ or caregiving quality.	Concurrent Mean (SD) All subjects: 11.4 (6.1) < 10: NR	Math score Achievement test Ages 6-8 yr	All 532 subjects 293 subjects <10	-0.17 (-0.28, -0.06) -0.42 (-0.92, 0.08)

FSIQ = Full-scale IQ, WISC-R = Wechsler Intelligence Scale for Children-Revised, WPPSI-R = Wechsler Preschool and Primary Scale of Intelligence-Revised, MDI = Mental Developmental Index, NR = Not reported, WRAT = Wide Range Achievement Test.

^aStudies are grouped by strength of study design, representativeness of the study population characteristics and blood Pb levels examined, and extent of consideration for potential confounding. There is not necessarily a continuum of decreasing strength across studies.

^bMost effect estimates are derived from linear models and are presented for a 1 µg/dL increase in blood Pb level.

^cResults not included in [Figure 4-15](#) because nonparametric analysis did not produce 95% CIs for various strata of blood Pb levels.

A few cross-sectional studies demonstrated larger Pb-associated decreases in cognitive function in groups with concurrent blood Pb levels <5 $\mu\text{g/dL}$ than groups with higher levels. Tellez-Rojo et al. (2006) estimated a larger decrement in age 2 year Bayley MDI per unit increase in blood Pb level for children with concurrent blood Pb levels <5 $\mu\text{g/dL}$ compared with children with blood Pb levels 5-10 $\mu\text{g/dL}$, and >10 $\mu\text{g/dL}$ (Figure 4-15 and Table 4-16). However, it is not clear what the implications of age 2 year MDI results may be on cognitive function at later ages. Among children ages 6-16 years participating in NHANES 1988-1994, Lanphear et al. (2000) found larger decrements in reading and math skills and memory per unit increase in blood Pb level in children with concurrent blood Pb levels <2.5 $\mu\text{g/dL}$ than children with levels <5 $\mu\text{g/dL}$, <7.5 $\mu\text{g/dL}$, <10 $\mu\text{g/dL}$, and all subjects. However, these children born 1972-1989 likely had higher blood Pb levels (Figure 3-18) and Pb exposures earlier in childhood, which may have contributed to the observed associations.

Several (Min et al., 2009; Jusko et al., 2008; Schnaas et al., 2006) but not all (Palaniappan et al., 2011) recent studies found evidence for a nonlinear blood Pb-cognitive function relationship in nonparametric regression analyses using splines or LOWESS with smoothing parameters that did not produce quantitative results for individual blood Pb groups. Similar to the pooled analyses of the seven prospective cohorts, these relationships were evaluated for a wide range of blood Pb levels. In the Rochester and Mexico City cohorts, the blood Pb-FSIQ relationship was more negative for children with lower blood Pb levels, specifically for peak blood Pb levels 2.1-10 $\mu\text{g/dL}$ (overall range: 2.1-46 $\mu\text{g/dL}$) in the Rochester cohort at age 6 years (Jusko et al., 2008) and for prenatal maternal week 28-36 blood Pb levels <6 $\mu\text{g/dL}$ (overall 5th-95th percentile 2.5-25 $\mu\text{g/dL}$) in the Mexico City cohort at ages 6-10 years (Schnaas et al., 2006). In a formal test of nonlinearity, Schnaas et al. (2006) found the nonlinear blood Pb term to fit the data better than a linear term. Among 278 children of age 4 years (blood Pb range: 1.3-24 $\mu\text{g/dL}$) who had high prenatal alcohol and drug exposure, Min et al. (2009) reported a p-value of 0.19 for a restricted cubic spline term for blood Pb level and described the covariate-adjusted concurrent blood Pb level-FSIQ curve to be more negative at blood Pb levels 1.3-7 $\mu\text{g/dL}$. Among 814 children in India ages 3-7 years, Palaniappan et al. (2011) mostly found linear associations between concurrent blood Pb level (range: 2.6-41 $\mu\text{g/dL}$) and indices of cognitive function. The exception was visual-motor skills, for which a greater blood Pb-associated decline was found with blood Pb levels >30 $\mu\text{g/dL}$. The linearity versus nonlinearity of the blood Pb-FSIQ concentration-response relationship within a lower, more narrow range of blood Pb levels has not been examined in detail.

Few studies of adults have examined whether the relationship between blood or bone Pb level and cognitive function is described better with a linear or nonlinear function. In

analyses of adults in NHANES, only log-linear models were used to fit the data ([Krieg et al., 2010](#); [Krieg and Butler, 2009](#); [Krieg et al., 2009](#)). Nonlinearity in the BMS and NAS cohorts was examined with the use of quadratic terms, penalized splines, or visual inspection of bivariate plots ([Bandeem-Roche et al., 2009](#); [Wang et al., 2007a](#); [Weisskopf et al., 2007b](#); [Shih et al., 2006](#)). There was some evidence for nonlinearity in prospective analyses of the NAS cohort ([Figure 4-7](#) and [Figure 4-8](#)), but not all results indicated greater declines in cognitive function per unit increase in bone Pb level in the lower bone Pb groups. Wang et al. ([2007a](#)) found that among NAS men with an HFE gene variant, there was a larger decline in MMSE score per unit increase in tibia Pb level at higher tibia Pb levels ([Figure 4-8](#)). In the BMS cohort, linear relationships were indicated for various tests of cognitive function by a statistically nonsignificant quadratic term ([Shih et al., 2006](#)) or spline ([Bandeem-Roche et al., 2009](#)) for tibia Pb level.

Although not specific to Pb exposure, attenuation of concentration-response relationships at higher exposure or dose levels has been reported in the occupational literature, and explanations have included greater exposure measurement error and saturation of biological mechanisms at higher levels and larger proportions of at-risk populations at lower exposure levels ([Stayner et al., 2003](#)). Hypotheses for nonlinearity in the relationship between Pb and cognitive function include a lower incremental effect of Pb due to covarying risk factors such as low SES, poor caregiving environment, higher exposure to other environmental risk factors ([Schwartz, 1994](#)), different mechanisms operating at different exposure levels, and confounding by omitted or misspecified variables. The contribution of these factors to the supralinear relationship observed between blood Pb levels and cognitive function in children has not been examined in many epidemiologic studies to date. Some studies found that risk factors such as SES, parental education, and parental caregiving quality explain a greater proportion of variance in cognitive function than does blood Pb level ([Wasserman et al., 1997](#); [Greene et al., 1992](#)). Recently, among 57,678 fourth grade children across North Carolina, Miranda et al. ([2009](#)) found that lower parental education and enrollment in a free/reduced fee lunch program accounted for larger decrements in EOG scores than did blood Pb level across the various quantiles of EOG score distribution ([Figure 4-6](#)). In a meta-analysis, the blood Pb-FSIQ relationship was more negative in studies of lower SES populations than in studies of higher SES populations ([Schwartz, 1994](#)).

Few studies have examined effect modification of the blood Pb level-cognitive function relationship by covarying risk factors such as sociodemographic factors, and the limited evidence is inconclusive. None of these studies examined effect modification within specific strata of blood Pb levels. Among children in the Boston cohort in the higher cord blood Pb level group ($>10 \mu\text{g/dL}$), cognitive function between ages 24 and 57 months increased in the group that was female and had higher HOME score, SES, and maternal

IQ ([Bellinger et al., 1990](#)). In the Port Pirie, Australia cohort, larger blood Pb-associated FSIQ decrements were found in groups with lower SES at age 11-13 years ([Tong et al., 2000](#)) but not lower HOME score at age 4 years ([McMichael et al., 1992](#)). Similar effects of blood Pb level on FSIQ were estimated in the higher SES Boston cohort and the lower SES Rochester cohort ([Table 4-3](#)). Overall, evidence does not clearly indicate whether the blood Pb-cognitive function relationship is modified by factors such as SES or other sociodemographic characteristics or whether these differences can explain the observed nonlinear concentration-response relationship.

Results from the pooled analysis by Rothenberg and Rothenberg ([2005](#)) do not indicate that residual confounding by covariates explains the nonlinear blood Pb-FSIQ relationship. Modeling maternal IQ, HOME score, maternal education, and birthweight as spline functions ($df = 2$) did not significantly improve model fit either with a linear blood Pb term or log blood Pb term, which indicated that the improved model fit with log-specification of blood Pb level was not influenced by the modeling of covariates as linear or nonlinear functions.

Bowers and Beck ([2006](#)) postulated that a supralinear concentration-response function necessarily will be found in a model with a log-normally distributed independent variable and a normally distributed outcome variable. However, as discussed in the 2006 Pb AQCD, this modeling strategy was not employed in the epidemiologic analyses showing a supralinear concentration-response function. FSIQ scores generally were not forced into a normal distribution. Normalized FSIQ scores were not the basis for individual findings from four of the seven studies included in the pooled analysis by Lanphear et al. ([2005](#)) or the results pooling the seven cohorts ([Hornung et al., 2006](#)). Further, a log-linear model (a linear relationship between IQ and the log of blood Pb) provided a better fit of the pooled data.

Results from prospective analyses in the Boston and Rochester cohorts for associations between blood Pb level as a continuous variable and FSIQ in groups of children in the lower segment of the population blood Pb distribution have not identified a threshold in the range of blood Pb levels examined. In the Boston cohort, higher age 2 year blood Pb levels were associated with lower FSIQ at age 10 years in children with blood Pb levels 1-9.3 µg/dL whose peak blood Pb levels were less than 10 µg/dL ([Bellinger, 2008](#); [Bellinger and Needleman, 2003](#)). Schwartz ([1994](#)) explicitly assessed evidence for a threshold in the Boston cohort data by regressing FSIQ and blood Pb level on covariates such as age, race, maternal IQ, SES, and HOME score and fitting a nonparametric smoothed curve to the residuals of each regression model (variation in FSIQ or blood Pb level not explained by covariates). A 7-point decrease in FSIQ was found over the range of blood Pb residuals below 0 (corresponding to the mean blood Pb level of 6.5 µg/dL),

indicating an association between blood Pb level and FSIQ well below the mean blood Pb level. Results from the Rochester cohort did not identify a threshold either. Higher concurrent blood Pb levels (range 0.5-8.4 µg/dL, detection limit = 1 µg/dL) were associated with lower FSIQ at ages 3 and 5 years in children with peak blood Pb levels <10 µg/dL ([Canfield et al., 2003a](#)). A threshold also was not identified for the association between concurrent blood Pb level and MDI score at age 2 years among children in Mexico City ([Téllez-Rojo et al., 2006](#)). Higher blood Pb level was associated with a lower MDI score in the group of children with blood Pb levels <5 µg/dL (range 0.8-4.9 µg/dL) ([Téllez-Rojo, 2008](#)). Other results based on nonparametric regression analyses did not identify a threshold for Pb-associated cognitive function decrements in children, but results have weaker implications because the blood Pb levels in the examined populations were higher (e.g., minimum 2.1 µg/dL, 5th percentiles 2.5 µg/dL) ([Jusko et al., 2008](#); [Schnaas et al., 2006](#); [Lanphear et al., 2005](#)) than those in most current U.S. children.

In conjunction with downward trends in population blood Pb distributions ([Figure 3-16](#)), more sensitive quantification methods have improved measurement of blood Pb levels in the lower portion of the population distribution (e.g., in NHANES, from 0.6 µg/dL in 1999-2002 to 0.025 µg/dL in 2003-2004). Consequently, the examination of groups of children (ages 4-15 years) with lower blood Pb levels, in the range of <1 to 16 µg/dL, has indicated Pb-associated cognitive function decrements or increases in externalizing behaviors at lower blood Pb levels ([Cho et al., 2010](#); [Kim et al., 2009b](#); [Miranda et al., 2009](#); [Braun et al., 2008](#); [Miranda et al., 2007a](#); [Braun et al., 2006](#)). In the studies examining concurrent blood Pb levels, the potential contribution of higher past Pb exposures obscures assessment of a threshold. For example, Braun et al. ([2008](#); [2006](#)) found higher odds of parental reports of conduct disorder and ADHD among children ages 4-15 years with concurrent blood Pb levels ~1.0 µg/dL compared with children with blood Pb levels <0.8 µg/dL (6 or 7% below detection limit of 0.3 µg/dL). However, children likely had higher early childhood blood Pb levels, and adolescents in the study were born in the 1970s during the use of leaded gasoline.

Results from Miranda et al. ([2009](#)) did not identify a threshold with the examination of blood Pb levels measured between ages 6 and 36 months during 1995-1999. Among 57,678 children in North Carolina, lower 4th grade EOG scores were found in children in the blood Pb level category of 2 µg/dL (exact values not reported) compared with children in the blood Pb level category of 1 µg/dL, which included children with blood Pb levels at or below the detection limit. Other analyses of blood Pb level as a categorical variable did not clearly address the identification of a threshold for the blood Pb-cognitive function relationship. However, these analyses of categories that encompass a wider range of blood Pb levels may be less sensitive. Cognitive function decrements

were limited to children ages 7-8 years in the U.K. with age 30-month blood Pb levels 5-10 µg/dL (compared with blood Pb levels 0-2 µg/dL) ([Chandramouli et al., 2009](#)) and children ages 6-10 years in New England with concurrent blood Pb levels 5-10 µg/dL (compared with blood Pb levels 1-2 µg/dL) ([Surkan et al., 2007](#)).

Some toxicological studies found nonlinear relationships between Pb exposure and effects related to impaired learning and memory in animals, although these differ from the nonlinear relationships found in epidemiologic studies of children in that nonlinearity in animal studies has been demonstrated as lower and higher Pb exposures having effects in opposite directions (U- or inverse U-shaped curves). Epidemiologic results for nonlinearity indicate negative effects over the entire blood Pb distribution examined. Results summarized across multiple studies in multiple species demonstrated that lower Pb exposures increased FI response rates relative to controls, and higher Pb exposures decreased FI response rates ([Cory-Slechta, 1994](#)). Increased FI response rates indicate impaired learning by reflecting the impaired ability of animals to respond according to a fixed schedule of reinforcement ([Section 4.3.2.3](#)). Consistent with previous findings, Rossi-George et al. ([2011](#)) found that 50 ppm lifetime Pb exposure increased FI overall rate in rats tested at age 2 months whereas 150 ppm Pb exposure did not affect FI responses. Nonlinear effects of Pb on learning were not observed with longer duration exposures (e.g., 8-11 months) ([Cory-Slechta, 1990](#)). The nonlinear effects of Pb on impaired learning were supported by evidence in animals indicating that lower and higher Pb exposures differentially activate underlying mechanisms. Gilbert et al. ([1999](#)) found reduced LTP magnitude in adult rats exposed to 1,000-5,000 ppm but not 10,000 ppm Pb acetate in drinking water from GD16. LTP is one indication of synaptic plasticity ([Section 4.3.10.4](#)), which is considered to be a major cellular mechanism underlying learning and memory. Synaptic plasticity also is affected by glutamatergic neurotransmission via its NMDA receptor ([Section 4.3.10.8](#)), and reduced glutamate release in the hippocampus was found in adult rats exposed to Pb acetate from GD15-GD16 with blood Pb levels 27-40 µg/dL but not with blood Pb levels of 62-117 µg/dL ([Lasley and Gilbert, 2002](#)).

Dopaminergic neurotransmission is involved in many CNS processes including cognition, behavior, and motor function. The shape of the Pb-DA concentration-response relationship varied among rodent studies. Some studies found that lower Pb exposures (e.g., 27, 50 ppm) did not affect or increased DA activity relative to controls and higher Pb exposure (109, 150, 250 ppm) ([Leasure et al., 2008](#); [Virgolini et al., 2005](#); [Lewis and Pitts, 2004](#)). However, sometimes in the same study, in other regions of the brain or under some conditions, higher Pb exposures (109, 150 ppm) increased or impaired DA activity ([Leasure et al., 2008](#); [Virgolini et al., 2005](#)). These differential responses of DA may be related to the diverse CNS effects of DA in different regions of the brain. For

example, the increased forebrain dopamine turnover with 27 ppm gestational/lactational Pb acetate exposure was accompanied by less spontaneous activity in male mice compared with male mice exposed to 109 ppm Pb ([Leasure et al., 2008](#)).

In vitro results indicated differential effects on calcineurin enzyme activity, with inhibited activity resulting from higher Pb exposure ($>2 \times 10^{-4}$ μM) and stimulated activity from lower Pb exposure ([Kern and Audesirk, 2000](#)). While calcineurin activity has been found to modulate learning, LTP, and behavior in animals, lower calcineurin activity has been associated with both impaired and improved performance on learning and memory tasks ([Zeng et al., 2001](#)). Thus, it is uncertain whether altered calcineurin activity can explain the nonlinear relationships observed between Pb exposure and impaired learning in animals. At lower concentrations, Pb may displace calcium at its binding sites on calmodulin and by acting as a calmodulin agonist at the catalytic A subunit of calcineurin stimulate calcineurin activity. At higher Pb exposure, Pb may bind directly to a separate calcium-binding B subunit, override the calmodulin-dependent effect and turn off the activity of calcineurin. Lasley and Gilbert ([2002](#)) found that 2,000 ppm but not 5,000 or 10,000 ppm Pb acetate exposure of rats (in drinking water starting at GD15-GD16) inhibited glutamate release by acting as a calcium mimetic.

Some toxicological studies have found nonlinear relationships for non-cognitive outcomes in animals. U-shaped Pb concentration-response relationships were found for spontaneous motor activity level and latency to fall from rotarod ([Leasure et al., 2008](#)). Inverted U-shaped relationships were found for hippocampal neurogenesis related to Pb exposure duration (24 hours not 28 days after BrdU injection) or concentration ([Fox et al., 2008](#); [Gilbert et al., 2005](#)). Evidence also points to differences in hormone production by Pb exposure concentration. In male mice with long-term Pb exposure (PND21-9 months of age), basal corticosterone levels were significantly lower with 50 ppm Pb than with 150 ppm Pb or controls ([Virgolini et al., 2005](#)). The visual system in animals also has shown to be affected differentially by lower versus higher Pb exposure (GD1-PND10, pup blood Pb levels 10-12, 24-27, and 42-46 $\mu\text{g/dL}$). Inverted U-shaped concentration-response curves were observed for rod photoreceptor numbers in the retina ([Giddabasappa et al., 2011](#)) and retinal layer thickness ([Fox et al., 2010](#)). These dichotomous histological findings may give insight to the complex Pb-associated changes in ERG wave amplitudes that vary by exposure timing and dose ([Section 4.3.6.2](#)).

To conclude, several studies found a supralinear blood Pb-cognitive function concentration-response relationship in children but not adults based on comparisons of effect estimates in lower and higher strata of blood Pb level and results from nonparametric regression modeling. Explanations for this supralinear relationship have not been well characterized by epidemiologic studies. Evidence from the prospective

studies in the Boston and Rochester cohorts has not identified a threshold for Pb-associated cognitive function decrements in the range of blood Pb levels examined. Increases in childhood blood Pb levels ranging from those at or below the detection limit of 1.0 µg/dL to 9.3 µg/dL (means: 3.3 and 3.8 µg/dL) were associated with cognitive function decrements at ages 3 to 10 years in children whose peak blood Pb levels were less than 10 µg/dL ([Bellinger, 2008](#); [Canfield, 2008](#); [Bellinger and Needleman, 2003](#); [Canfield et al., 2003a](#)). Further, a recent study found an association between higher blood Pb levels measured at ages 6-36 months (1995-1999) and lower 4th grade EOG scores in 57,678 children in North Carolina with blood Pb levels at or below the detection limit of 1 µg/dL to 16 µg/dL ([Miranda et al., 2009](#)). Concurrent blood Pb levels in the range of 0.8-9.8 µg/dL (detection limit not reported) were associated with MDI decrements in children age 2 years in Mexico City ([Téllez-Rojo, 2008](#); [Téllez-Rojo et al., 2006](#)). The lack of a reference population with blood Pb levels reflecting pre-industrial Pb exposures limits the ability to identify a threshold. Analysis of ancient bones in pre-industrialized societies suggests that “background” blood Pb levels in pre-industrial humans may have been more than 50-fold lower than those of the current U.S. population ([Flegal and Smith, 1992](#)) and lower than the levels at which neurodevelopmental effects have been examined. Thus, the current evidence does not preclude the possibility of a threshold for neurodevelopmental effects in children existing with lower blood levels than those currently examined. While distinct from supralinear relationships observed in epidemiologic studies, some toxicological studies showed that lower Pb exposures (e.g., 50 ppm in drinking water) induced learning and memory impairments in animals compared to control exposures or higher Pb exposures (e.g., 150 ppm). Additional toxicological evidence suggests that differentially activated mechanisms at lower and higher Pb exposures such as reduced LTP and hippocampal glutamate release with lower Pb exposures may provide explanation for the impaired learning and memory observed with lower Pb exposures in animals.

4.3.13 Confounding in Epidemiologic Studies of Nervous System Effects

In addition to Pb exposure, many factors influence cognitive function and behavior in children, including parental IQ and education, SES of the family, quality of the caregiving environment, and other environmental exposures ([Wasserman and Factor-Litvak, 2001](#)). These other risk factors often are correlated with blood, tooth, and bone Pb levels, thus, a major challenge to observational studies examining associations of Pb biomarker levels with cognitive function and behaviors in children has been the assessment and control for potential confounding factors. By definition, a confounder is associated with both the independent variable and the outcome and consequently has the

potential to bias the association between the independent variable of interest and the outcome. Most epidemiologic studies of Pb biomarkers in children have examined potential confounding by parental IQ and SES-related variables such as parental education, household income, and the Hollingshead Four-Factor Index of Social Position, which incorporates education and income of both parents. Fewer but still several studies have examined confounding by quality of the caregiving environment (i.e., HOME score), birth weight, and smoking exposure. A relatively smaller number of studies have considered nutritional status, other environmental exposures, parental substance abuse, or parental psychopathology. Studies have varied with respect to the number of potential confounding factors examined, with some studies considering multiple SES-related factors and other studies focusing on a smaller set. The extent of confounding by a particular factor likely varies across studies, depending on the population examined. Thus, the impact of adjustment for specific covariates on the Pb effect estimate also likely varies across studies.

Various methods have been used to control for potential confounding: examining a population relatively homogeneous in SES, examining populations in which factors are not correlated, conducting multivariate regression, characterizing the change in the Pb biomarker effect estimate with adjustment for a covariate, and examining associations in different strata of a covariate. The evidence derived from each of these control strategies is discussed below. No single method is without limitation, and adjustment for SES is difficult as it is highly correlated with Pb exposure and there is no single variable that fully represents SES. Residual confounding also is likely by factors not considered. The combination of evidence from prospective studies that considered several well-characterized potential confounding factors plus evidence that Pb exposure impairs cognitive function and behavior in animals, in particular, visual-spatial memory, executive function, and response inhibition, which are also found to be affected in children, increase confidence that the associations observed between Pb biomarker levels and neurodevelopmental effects in children represent a relationship with Pb exposure.

In the Boston prospective study, potential confounding by SES was reduced by study design and statistical adjustment for SES. The study subjects were from middle- to upper-middle-class families, a majority with married, college-educated parents. Hence, the potential for confounding by SES in this study was considerably less compared to other studies examining similar outcomes. In this cohort, higher prenatal cord and concurrent blood Pb levels were associated with FSIQ decrements at age 57 months, and higher age 2 year and age 57 month blood Pb levels were associated decrements in FSIQ and executive function at age 10 years ([Stiles and Bellinger, 1993](#); [Bellinger et al., 1992](#); [Bellinger et al., 1990](#)). In contrast, blood Pb levels measured at various ages were weakly associated with cognitive function decrements at age 7 years in the Sydney, Australia

cohort of middle-SES children (i.e., 20% mothers with > high school education) ([Cooney et al., 1991](#)). However, a relationship between Pb exposure and cognitive function decrements is supported by a similar magnitude of blood Pb-associated FSIQ decrement found in the Boston and the low-SES Rochester cohort (majority of mothers with < college education and annual income <\$15,000), with adjustment for similar covariates ([Figure 4-2](#) and [Table 4-3](#)) ([Canfield et al., 2003a](#)). For some outcomes, larger effects were estimated in the Boston cohort than in other cohorts.

Blood Pb levels also were associated with cognitive function decrements in populations in which higher blood Pb levels were not correlated with lower SES ([Factor-Litvak et al., 1999](#); [Bellinger et al., 1987](#)). In the Yugoslavia cohort, blood Pb levels at age 4 years were higher in groups with higher maternal education, maternal IQ, and HOME score in the city near Pb sources and were lower in the distant city. Among all children, higher blood Pb level was associated with lower FSIQ and learning and memory scores and with higher ratings of internalizing behaviors ([Factor-Litvak et al., 1999](#)). In the Boston cohort, parental education, social class, HOME score, and birth outcomes were similar among low (<3 µg/dL), medium (6-7 µg/dL), and high (≥ 10 µg/dL) cord blood Pb level groups. Further, adjusting for these and other demographic factors, Bellinger et al. ([1987](#)) found that children in the high cord blood Pb group had a 4.8-point lower Bayley MDI score at age 2 years than did children in the low cord blood Pb group.

The primary method used by epidemiologic studies to control for potential confounding, in particular recent studies of children with blood Pb levels more similar to current U.S. levels, has been multivariate regression. Some studies modeled a set of covariates based on a priori evidence, whereas others selected specific covariates based on their association with the outcome in a model with all potential covariates and/or a greater than 10% change in the blood Pb level effect estimate. Studies also varied in the number of potential confounding factors included in models. Some included multiple SES-related variables, whereas others analyzed one or two factors. Regardless of the method used to select model covariates or the number of covariates included, studies consistently found associations of higher blood Pb level with cognitive function decrements and poorer behavior. The evidence suggests that confounding by particular factors may vary across populations and increases confidence that the associations observed with neurodevelopmental effects in children represent a relationship with Pb exposure.

The consistency in finding associations across populations with different SES and co-exposures and across studies examining different covariates was reinforced in pooled and meta-analyses ([Marcus et al., 2010](#); [Lanphear et al., 2005](#); [Schwartz, 1994](#)). Pooling data from seven international prospective cohorts, Lanphear et al. ([2005](#)) found similar FSIQ decrements per log increase in blood Pb level (-2.6% to +8.6% difference) by

excluding one study at a time. These results indicated a relatively robust pooled estimate despite between-study differences in population characteristics, including SES. In a meta-analysis, Schwartz ([1994](#)) found a relatively narrow range of blood Pb-FSIQ effect estimates among studies despite large between-study differences in the correlation between blood Pb level and SES. A wider range of effect estimates would be expected if omitted SES factors confounded the association. A recent meta-analysis of the association between blood Pb level and conduct problems from earlier and recent studies of children found that adjustment for SES and home environment did little to attenuate the association ([Marcus et al., 2010](#)).

Among the several studies that provided both unadjusted and adjusted effect estimates, most indicated that blood Pb level was a statistically significant predictor of cognitive function (e.g., FSIQ, executive function, learning, memory) in children ages 5-10 years before and after adjusting for potential confounders. Although most effect estimates changed by 20-50% in multivariate models, most remained within the 95% CI of the unadjusted estimate ([Min et al., 2009](#); [Kordas et al., 2006](#); [Schnaas et al., 2006](#); [Canfield et al., 2003a](#); [Dietrich et al., 1993b](#); [Bellinger et al., 1992](#)). Such observations were made in previous analyses of the Boston and Rochester cohort with mean blood Pb levels 6.5 and 5.8 µg/dL, respectively, with adjustment for SES, maternal IQ and education, and HOME score ([Canfield et al., 2003a](#); [Bellinger et al., 1992](#)). These analyses also adjusted for or considered potential confounding by indicators of nutritional status. Recent studies of children of a similar age range and mean blood Pb levels also found statistically significant associations (as indicated by correlation and/or regression coefficients) between blood Pb level and cognitive function before and after adjustment for similar covariates ([Min et al., 2009](#); [Chiodo et al., 2007](#)); however, because of the high prevalence of prenatal alcohol or drug use in these populations, their results may be less generalizable than results from the Rochester and Boston cohorts.

Blood Pb level also was a statistically significant predictor of cognitive function after adjustment for covariates such as maternal education and IQ, SES, and HOME score in with children with higher mean blood Pb levels, 8-14 µg/dL ([Kordas et al., 2006](#); [Schnaas et al., 2006](#); [Tong and Lu, 2001](#); [Dietrich et al., 1993b](#)). Exceptions include multiple analyses of the Cleveland cohort, in which blood Pb level was estimated to have a weak and imprecise or null effect after adjustment for potential confounding factors ([Greene et al., 1992](#); [Ernhart et al., 1989](#); [Ernhart et al., 1988](#)). Analyses of the Cleveland cohort considered similar potential confounding factors as other studies. HOME score was the major factor accounting for the attenuation of the Pb effect estimate in the Cleveland cohort. An analysis of the Yugoslavia cohort, which adjusted for most of the same covariates as the Cleveland analyses reported larger magnitude blood Pb-cognitive function associations in covariate-adjusted models ([Factor-Litvak et al., 1999](#)). The

collective findings in children indicate potential confounding by the SES-related and demographic factors examined in the literature base but also demonstrate that blood Pb level is an independent predictor of cognitive function decrements in children with adjustment for these factors.

A challenge to separating the effects of Pb exposure from those related to SES and parental caregiving quality is their frequently high correlation with blood Pb levels. In such cases, it is difficult to know how much variation in the outcome to attribute to each of the risk factors ([Needleman and Bellinger, 2001](#)). For example, due to the high correlation between blood Pb level and SES, a model that includes SES may underestimate the Pb effect because some of the variance in outcome due to Pb is mistakenly attributed to the variance due to SES. This misattribution may be exacerbated when multiple correlated variables are included in the same model (i.e., overcontrol). The relationships observed for Pb biomarker levels with SES and parental caregiving quality may indicate that they are proxies or determinants of Pb exposure rather than a confounder of the association of interest. Lower SES in urban children is closely linked to residence in older, poorer condition housing that, in turn, may increase exposure of children to environmental Pb and risk of cognitive function decrements ([Clark et al., 1985](#)). In such cases where SES is a determinant of Pb exposure, statistical adjustment for SES will result in overcontrol of the Pb effect ([Bellinger, 2004a](#)). This type of overcontrol could apply to results from the New Zealand cohort, which were adjusted for residence in older wooden housing, which is associated with higher exposure to Pb paint and accumulated dust and soil and higher child tooth Pb levels ([Fergusson et al., 1988a, b](#)). However, even in models with older wooden housing, Pb remained a statistically significant predictor of poorer reading skills and teacher ratings of school performance. SES has been shown to be an effect modifier of the Pb-child cognitive function relationship in some populations. Larger blood Pb-associated decreases in cognitive function were found with lower SES in some studies ([Ris et al., 2004](#); [Tong et al., 2000](#); [Bellinger et al., 1990](#)) and higher SES in a meta-analysis ([Schwartz, 1994](#)). In cases of effect modification, potential confounding by SES is less likely.

In summary, the collective epidemiologic evidence consistently demonstrates associations of higher blood and tooth Pb levels with cognitive function decrements and poorer behavior in children. These associations have been observed in diverse populations in the U.S., Mexico, Europe, Asia, and Australia. Associations have been observed across studies that use different methods to control for confounding and adjust for different potential confounding factors but commonly, maternal IQ and education, SES, and HOME score. Several studies have found associations with additional adjustment for smoking exposure, child birth outcomes, and nutritional factors. No single method to control for potential confounding is without limitation, and there is potential

for residual confounding by unmeasured factors. However, the consistency of findings among different populations and study methods with consideration of several well-characterized potential confounding factors as described above increases confidence that the associations observed between Pb biomarker levels and neurodevelopmental effects in children represent a relationship with Pb exposure. Biological plausibility is provided by the coherence with extensive evidence in animals with Pb exposures that produce blood Pb levels relevant to this ISA and that is not subject to confounding by factors such as social class and correlated environmental factors. Further, Pb exposure has been shown to induce decrements in visual-spatial memory, rule learning and reversal, and response inhibition, which also have been associated with blood or tooth Pb levels in children. Additional support for the epidemiologic evidence is provided by extensive toxicological evidence describing modes of action for Pb-induced cognition and behavioral problems, including changes in neurogenesis, synaptic pruning, and neurotransmitter function in the hippocampus, prefrontal cortex, and nucleus accumbens of the brain ([Section 4.3.10](#)).

4.3.14 Public Health Significance of Associations between Pb Biomarkers and Neurodevelopmental Effects

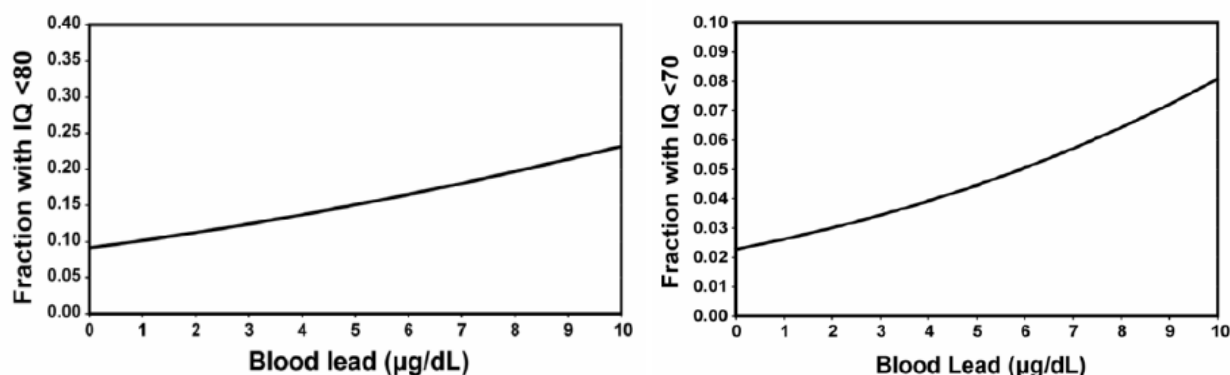
As described in [Section 4.3.2.1](#), most studies found that a 1 µg/dL increase in blood Pb level was associated with decrements in FSIQ in school-aged children in the range of <1 to 2 points, depending on the model and blood Pb level range examined ([Figure 4-2](#) and [Table 4-3](#)). Similarly, a 1 µg/dL increase in blood Pb level typically was associated with lower scores on tests of executive function ([Table 4-8](#)) and academic performance ([Table 4-9](#)), and higher ratings of behavioral problems ([Figure 4-9](#), [Table 4-11](#), [Table 4-12](#)) on the order of less than 1 standard deviation. Such findings prompt consideration of the public significance of blood Pb level-associated effects on decrements in cognitive function and behavior in children, specifically, whether the magnitudes of change have consequences on the health and life-success of individuals. According to the WHO, “Health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity” ([WHO, 1948](#)). By this definition, even decrements in health status that are not severe enough to meet diagnostic criteria might be undesirable if they reflect a decrement in the well-being of an individual. Also, decreases in health indices or life-success may not be observable except at the population level. The American Thoracic Society discussed the need to consider the prevalence of exposures in the population and exposure to other risk factors in evaluating whether shifts in the population-level risk are adverse ([ATS, 2000](#)). Neurodevelopmental decrements measured in childhood may set affected children on trajectories more prone toward lower educational attainment and financial well-being. Thus, early effects in children may have lifetime consequences. There also may be groups in the population at increased risk of

neurodevelopmental effects from Pb exposure. For example, some evidence points to larger blood Pb-associated decrements in cognitive function in children with lower SES ([Ris et al., 2004](#); [Tong et al., 2000](#); [Bellinger et al., 1990](#)), whereas a meta-analysis found a larger effect estimate for studies with higher SES ([Schwartz, 1994](#)).

It has been argued that blood Pb-associated decrements in IQ points less than 3 or 4 points are meaningless given that such changes are within the standard error of a single test (i.e., the statistic that defines the range within which the true value of an individual is likely to lie) ([Kaufman, 2001](#)). However, this argument incorrectly assumes that conclusions drawn from individual-level data apply to populations. Evidence does not indicate that the standard error is nonrandom, i.e., biased in one direction. Hence, there is no reason to expect that children with higher blood Pb levels systematically test lower than their true IQ value or that children with lower blood Pb levels test higher than their true IQ value. Thus, in a population of children, on a given assessment, some children will test lower than their true value and others will test higher than their true value. In such cases, between-group differences will be measureable on a population basis. Error in the measurement of IQ in an individual will contribute nondifferential error on a population-level and make it more difficult to detect an association if one exists.

The issue of individual-level versus population-level risk also pertains to the implications of the magnitude of decrease in cognitive function or increase in behavioral problems per unit increase in blood Pb level. Although fractional changes in FSIQ, memory, or attention may not be consequential for an individual, they may be consequential on a population level, especially in the two tails of the distribution ([Bellinger, 2007, 2004b](#)). For example, interventions that shift the population mean, in a beneficial direction, by an amount that is without clinical consequence for an individual have been shown to produce substantial decreases in the percentage of individuals with values that are clinically significant ([Bellinger, 2007, 2004b](#)). In statistical exercises not specific to Pb or analysis of data collected from individuals, Weiss ([1990, 1988](#)) predicted that a downward shift of five points in mean IQ, if the amount of dispersion in the distribution remained the same, should be accompanied by a doubling of the numbers of individuals with scores two or more standard deviations below the mean. In this hypothetical model, with a reduction in population mean IQ from 100 to 95, the percentage of individuals predicted to score above 130 (two standard deviations above the mean) decreases from 2.3% to 0.99%. Weiss ([1988](#)) stated that the implication of such a loss transcends the current circumscribed definitions of risk. In addition to implications on the loss of intellectual ability, the loss of a few IQ points potentially could result in the loss of academic opportunities in children. For example, schools or programs for the gifted have used IQ cut-offs (e.g., score of 130) to screen or accept applicants. In another hypothetical analysis presented in the 2006 Pb AQCD ([U.S. EPA, 2006b](#)), based on a

blood Pb-FSIQ effect estimate of -0.9 points/ $\mu\text{g/dL}$ (the median effect estimated for blood Pb levels $<10 \mu\text{g/dL}$), the fraction of the population with an FSIQ <80 is predicted to more than double from 9% with a blood Pb level of $0 \mu\text{g/dL}$ to 23% with a blood Pb level of $10 \mu\text{g/dL}$ (Figure 4-16). The proportion with an FSIQ <70 , which often requires community support to live (WHO, 1992), is predicted to increase from a little over 2% with a blood Pb level of $0 \mu\text{g/dL}$ to about 8% with a blood Pb level of $10 \mu\text{g/dL}$ [(Figure 4-16) and (U.S. EPA, 2006b)]. These theoretical exercises estimate that for an individual in the low range of the IQ distribution, a Pb-associated decline of a few points might be sufficient to drop that individual into the range associated with increased risk of educational, vocational, and social failure.



Note: The results presented are based on a theoretical analysis of changes in population FSIQ using a concentration-response estimate of -0.9 FSIQ points/ $\mu\text{g/dL}$, which was the median estimate from studies reviewed in the 2006 Pb AQCD for blood Pb levels $<10 \mu\text{g/dL}$. The analysis estimates increasing proportions of individuals with FSIQ <80 and <70 with increasing blood Pb level. Source: 2006 Pb AQCD (U.S. EPA, 2006b).

Figure 4-16 Hypothetical effect of increasing blood Pb level on the proportion of the population with FSIQ <80 and <70 points.

The hypothetical predictions presented in Weiss (1990, 1988) and the 2006 Pb AQCD (U.S. EPA, 2006b) have been supported by findings from analyses using data from studies of blood Pb levels and FSIQ decrements in children. Among children in 1st and 2nd grades from towns around Boston, MA, Needleman et al. (1982) found that a 4-point downward shift in the study population mean verbal IQ estimated for tooth Pb levels $>24 \text{ ppm}$ was associated with a 3-fold increase in the percentage of children with a verbal IQ of <80 and a decrease in the percentage achieving a verbal IQ >125 from 5% to 0%.

The aforementioned hypothetical analyses and those using data collected from individuals that estimate Pb-associated changes in the population IQ distribution assume that the magnitude of change is equal across segments of the IQ distribution. Few studies of Pb and cognitive function have examined whether the effect of Pb varies across the distribution of cognitive function. However, in a recent study of fourth graders across the entire state of North Carolina, Miranda et al. (2009) found that higher blood Pb level measured once in each child between age 9 months and 3 years was associated with larger decreases in fourth grade EOG scores in the lower segment of the EOG distribution. An increase in blood Pb level from 1 to 10 µg/dL was estimated to decrease EOG score by 0.8 points in the 95th percentile of EOG scores but by 2.3 points in the 5th percentile of EOG score (Figure 4-6). These findings by Miranda et al. (2009) based on analysis of a large database representative of fourth graders in North Carolina indicate that a shift in the population mean from increased Pb exposure may increase the proportions of children at the lower end of the cognitive function distribution over that estimated by theoretical analyses.

In summary, the public health significance of evidence demonstrating associations between increases in blood Pb levels and decrements in IQ of children in the range of a few points is supported by hypothetical predictions that a shift in the population mean increases the proportion of individuals in the lower range of cognitive function and decreases the proportion of individuals in the upper range of cognitive function. These changes in the population distribution also were found in children in 1st and 2nd grade in Massachusetts in whom higher tooth Pb level was associated with decrements in IQ (Needleman et al., 1982). Results from a few studies suggested that the public health significance of decrements in cognitive function associated with higher blood Pb levels may be greater in certain groups of children, for example, those in the lower range of cognitive function (Miranda et al., 2009) and those in lower SES groups (Ris et al., 2004; Tong et al., 2000; Bellinger et al., 1990). On a population-level, small Pb-associated decreases in cognitive function could increase the number of individuals at increased risk of educational, vocational, and social failure and decrease the number of individuals with opportunities for academic and later-life success.

4.3.15 Summary and Causal Determination

The collective body of epidemiologic and toxicological evidence integrated across that reviewed in the 2006 Pb AQCD (U.S. EPA, 2006b) and recent studies indicates relationships between Pb exposure and a range of nervous system effects. In children, effects on cognitive function include FSIQ, learning, memory, executive function, and academic performance. Externalizing behaviors in children were evaluated in two groups:

1) attention, impulsivity, hyperactivity, and ADHD; and 2) conduct disorders which included aggression, delinquency, and criminal offenses. Effects on internalizing behaviors include withdrawn behavior and symptoms of depression, fearfulness, and anxiety. Other nervous system effects evaluated in children are auditory function, visual function, and motor function. Relationships for Pb exposure with cognitive function, auditory function, and visual function also were evaluated in adults. Other nervous system effects in adults examined in relation to Pb exposure include those related to psychopathological effects such as symptoms of depression, anxiety, and panic disorder. Additionally, effects on neurodegenerative diseases include Alzheimer's disease, ALS, Parkinson's disease, and essential tremor. The subsequent sections describe the evaluation of evidence for each of these outcome groups with respect to causal relationships with Pb exposure using the framework described in [Table II](#) of the [Preamble](#). The application of key evidence, supporting or contradictory, to the causal framework is summarized in [Table 4-17](#).

4.3.15.1 Evidence for Cognitive Function Decrements in Children

A causal relationship between Pb exposure and cognitive function decrements in children is supported by several lines of evidence: 1) findings from prospective studies in diverse populations consistently demonstrating associations of higher blood and tooth Pb levels with lower FSIQ and performance on tests of executive function and academic performance in children ages 4-17 years and young adults ages 18-20 years ([Section 4.3.2](#)); 2) coherence with evidence in animals for impairments in learning, memory, and executive function with relevant Pb exposures; and 3) evidence describing modes of action ([Table 4-17](#)).

Clear support for Pb-associated cognitive function decrements in children, as described in the 2006 Pb AQCD ([U.S. EPA, 2006b](#)), was provided by prospective epidemiologic studies indicating associations of higher earlier childhood (e.g., prenatal maternal or cord, age 2 or 4 year), concurrent (ages 4-10 year), and childhood average blood and tooth Pb levels with lower FSIQ in children ages 4-17 years ([Table 4-17](#) and [Section 4.3.2.1](#)). Evidence for such Pb biomarker metrics better characterized the temporal sequence between Pb exposure and decrements in cognitive function than did cross-sectional analyses. Across studies, FSIQ was measured with various instruments (i.e., WISC-R, WISC-III, WPPSI, Stanford-Binet) that were similar in scoring scale and measurement error. Associations were found in most of the prospective studies, conducted in the U.S., Mexico, Europe, and Australia in representative populations, most of which had moderate to high follow-up participation without indication of selective participation among children with higher blood Pb levels and lower cognitive function ([Table 4-3](#)). Another

strength of the evidence from prospective studies was the consideration for several potential confounding factors. As indicated in [Table 4-3](#), results from most cohort studies were adjusted for maternal IQ and education, child sex and birth weight, SES, and HOME score. Although not considered as frequently, some studies also found associations with adjustment for parental smoking, birth order, and nutritional factors. The consistency and reproducibility of the blood Pb-FSIQ association in children were demonstrated further in pooled analyses of seven prospective studies ([Lanphear et al., 2005](#); [Rothenberg and Rothenberg, 2005](#)) as well as multiple meta-analyses that combined results across various prospective and cross-sectional studies ([Pocock et al., 1994](#); [Schwartz, 1994](#); [Needleman and Gatsonis, 1990](#)), with Schwartz (1994) demonstrating the robustness of evidence to potential publication bias. These pooled or meta-analyses combine data that are somewhat heterogeneous in the tests used to assess FSIQ and the ages of blood Pb and FSIQ examined which could be sources of non-differential measurement error. Heterogeneity in the potential confounding factors examined and the method of assessment could produce residual confounding.

Among individual studies, a wide range of blood Pb-FSIQ effect estimates was obtained, which is not unexpected given the wide range of blood Pb levels examined and modeling methods used (i.e., linear, log-linear). The pooled analysis of seven prospective cohorts demonstrated precision of effect estimates by applying a uniform method across populations ([Lanphear et al., 2005](#)). A narrow range of estimates was obtained by excluding one study at a time, -2.4 to -2.9 points per log increase in concurrent (ages 4.8-10 years) blood Pb level. Results from several individual studies, in particular prospective studies, indicated a supralinear concentration-response relationship which estimated a greater decrement in cognitive function per unit increase in blood Pb level among children in lower strata of blood Pb levels than children in higher strata of blood Pb levels ([Figure 4-15](#) and [Table 4-16](#)).

Among the largest effect estimates were those found in the Boston and Rochester cohorts ([Canfield et al., 2003a](#); [Bellinger et al., 1992](#)), which had relatively smaller sample sizes but considered several potential confounding factors as listed above and in [Table 4-3](#), examined lower blood Pb levels than did other prospective studies, and examined cohorts that differed in SES. Thus, their results may be more representative of the effects of Pb exposure on FSIQ. In the Boston cohort, a 1 µg/dL increase in age 2 year blood Pb level was associated with a -1.6 (95% CI: -2.9, -0.2) point change in FSIQ at age 10 years in 48 children with age 2-year blood Pb levels 1-9.3 µg/dL (detection limit not reported) and peak blood Pb levels <10 µg/dL ([Bellinger, 2008](#); [Bellinger and Needleman, 2003](#)). In the Rochester cohort, a 1 µg/dL increase in concurrent blood Pb level was associated a -1.8 (95% CI: -3.0, -0.60) change in FSIQ at age 5 years in 101 children with concurrent blood Pb levels 0.5-8.4 µg/dL (detection limit = 1 µg/dL) and peak blood Pb levels

<10 µg/dL ([Canfield, 2008](#); [Canfield et al., 2003a](#)). In the pooled analysis, a 1 µg/dL increase in concurrent blood Pb level was associated a -2.9 (95% CI: -5.2, -0.71) change in FSIQ at ages 4.8-10 years in 103 children with a mean concurrent blood Pb level of 3.2 µg/dL and peak blood Pb levels <7.5 µg/dL ([Hornung, 2008](#); [Lanphear et al., 2005](#)). Results from the Boston and Rochester cohorts can be considered particularly informative because within each cohort, there was greater homogeneity in the tests used to assess FSIQ, the age of examination, and the methods used to measure potential confounding factors. Further, in Lanphear et al. ([2005](#)), 80% of the data for the lower portion of the blood Pb distribution was provided by the Boston and Rochester cohorts. These observations do not identify a threshold in the ranges of blood Pb level examined.

Null or weak associations were limited to a few cohorts, namely, the Cleveland and Sydney cohorts ([Greene et al., 1992](#); [Cooney et al., 1991](#); [1989a, b](#); [Ernhart et al., 1988](#)). The Cleveland and Sydney studies were not outliers with respect to population mean blood Pb levels or the specific confounding factors considered ([Table 4-3](#)), and the Cleveland cohort had high prevalence of maternal prenatal substance abuse which may limit the representativeness of results.

Other previous studies estimated smaller magnitude of effects for the blood Pb-FSIQ association in children ages 4-13 years with either linear or log-linear models but examined higher blood Pb levels (means: 7.8-16 µg/dL) and did not analyze the concentration response at the lower range of the study population blood Pb distribution ([Table 4-3](#)). Most recent cross-sectional studies supported associations between higher concurrent blood Pb levels and decrements in FSIQ. Some found associations in populations of children ages 6-11 years with lower blood Pb levels than other studies, i.e., means 1.7-5.0 µg/dL ([Table 4-17](#)). In exception, among children ages 6-10 years in New England with a mean concurrent blood Pb level 2.2 µg/dL, lower FSIQ was found in the group with blood Pb levels 5-10 µg/dL ([Surkan et al., 2007](#)). In many of these studies, the potential influence of higher past Pb exposures cannot be excluded. Although Zailina et al. ([2008](#)) had limited consideration for potential confounding, results from most of these studies were adjusted for SES and parental IQ or education. Chiodo et al. ([2007](#)) found an association with extensive consideration for potential confounding, including parental caregiving quality and prenatal drug exposure, which was prevalent in the study population.

A causal relationship between Pb exposure and cognitive function decrements in children also is supported by previous prospective studies (several of which contributed to the FSIQ evidence) that found associations of blood or tooth Pb level with decrements in executive function and academic performance in populations ages 4-20 years ([Sections 4.3.2.4, 4.3.2.5, and Table 4-17](#)). The bodies of evidence for executive function, and

academic performance are smaller than that for FSIQ but consistently indicate associations with blood or tooth Pb level. Associations with performance on tests of learning and memory were less consistently found across populations ([Section 4.3.2.3](#)). In most studies, previous and recent, multiple testing was common; however, the consistent pattern of association observed across the ages of blood Pb level and/or cognitive test examined increases confidence that the evidence is not unduly biased by an increased probability of associations found by chance alone. While recent studies of executive function and academic performance adjusted for SES and parental education and/or cognitive function, few examined potential confounding by parental caregiving quality.

Adding to the evidence for Pb-associated cognitive function decrements in children were recent prospective studies that indicated associations between higher earlier childhood blood Pb level and poorer academic performance in school-aged children ([Chandramouli et al., 2009](#); [Miranda et al., 2009](#)). Among 57,678 children in North Carolina, lower fourth grade EOG scores were found in children with age 9-36 month blood Pb levels categorized as 2 µg/dL compared with children with blood Pb levels categorized as 1 µg/dL (detection limit = 1 µg/dL), with adjustment for sex, race, school type, age of blood Pb measurement, parental education, and enrollment in a free lunch program as an indicator of SES ([Miranda et al., 2009](#)). Results from this study also indicated a greater incremental association of blood Pb level with decrement in EOG score among children in the lower end of the EOG distribution. Chandramouli et al. (2009) found lower school achievement tests scores in 488 children age 7 years in U.K. in the children with age 30 month blood Pb levels >5 µg/dL. These results were adjusted for several potential confounding factors, including SES, parental education, SES, home facilities score, and family adversity. Recent cross-sectional studies conducted in the U.S. ([Krieg et al., 2010](#); [Surkan et al., 2007](#)) found associations of concurrent blood Pb level with decrements in executive function and academic performance in children, including the large analysis of >700 children ages 12-16 years participating in NHANES ([Krieg et al., 2010](#)). Cho et al. (2010) did not find an association between concurrent blood Pb level and executive function among children in Korea ages 8-11 years with a mean blood Pb level 1.9 µg/dL.

Several studies found associations of higher prenatal, earlier infancy (e.g., age 1 year), and concurrent blood Pb levels with lower Bayley MDI scores in children ages 2 and 3 years ([Table 4-4](#)). Similar to studies of FSIQ, Tellez-Rojo et al. (2006) estimated a larger decrement in age 2 year MDI per unit increase in concurrent blood Pb level for children in Mexico City with blood Pb levels <5 µg/dL compared with children with blood Pb levels 5-10 µg/dL, and >10 µg/dL ([Figure 4-15](#) and [Table 4-16](#)). MDI is a well-standardized measure of current infant mental development. However, the test of MDI is not an intelligence test, and MDI scores, particularly before ages 2-3 years, are not

necessarily strongly correlated with later measurements of FSIQ in children with normal development.

A causal relationship between Pb exposure and cognitive function decrements in children is further supported by consistent observations in rodents and monkeys of decrements in learning, associative ability, memory, and executive function with relevant dietary Pb exposures. In particular, coherence was found between evidence in children and animals of Pb-associated decrements in visual-spatial memory, working memory ([Section 4.3.2.3](#)) and rule learning and reversal ([Section 4.3.2.4](#)). Previous studies in monkeys ages 5-10 years demonstrated impairments in learning, memory, and executive function with dietary Pb exposures during infancy only, lifetime after early infancy, and lifetime from birth that produced blood Pb levels of 15-36 µg/dL, some relevant to this ISA ([Rice, 1992b](#); [Rice and Gilbert, 1990a](#); [Rice, 1990](#); [Rice and Gilbert, 1990b](#); [Rice and Karpinski, 1988](#); [Gilbert and Rice, 1987](#)).

Several recent toxicological studies added to the evidence for impaired learning, associative ability, and memory in animals with blood Pb levels, 10-25 µg/dL after gestational-lactational, lactational, or lifetime (with and without gestational) Pb exposure ([Cory-Slechta et al., 2010](#); [Niu et al., 2009](#); [Stangle et al., 2007](#)). Together, the prospective epidemiologic and toxicological studies provide evidence for the temporal sequence between Pb exposure and decrements in cognitive function. Additional biological plausibility for Pb-associated cognitive function decrements was provided by toxicological evidence for the effects of Pb on modes of action for cognitive function ([Section 4.3.10](#)). Pb has been shown to increase the permeability of the blood-brain barrier and deposit in the target CNS. Pb has been shown to impair neurogenesis, synaptic architecture, and neurite outgrowth. The high activity of these processes during fetal and infant development provides biological plausibility for the effects of childhood Pb exposure on decrements in cognitive function. Cognitive function is mediated by the cortical and subcortical structures of the brain that integrate function in the hippocampus, prefrontal cortex, and nucleus accumbens using dopamine and glutamate as primary neurotransmitters. Experimental studies have shown that Pb induces changes in dopamine and glutamate release in these regions and decreases the magnitude of LTP, which is a major cellular mechanism underlying synaptic plasticity and learning and memory.

With regard to critical lifestages and time periods of Pb exposure, toxicological evidence clearly demonstrates impaired learning and memory in animals exposed to Pb gestationally with or without lactational exposure. This evidence is well supported by knowledge that processes such as neurogenesis, synaptogenesis, and synaptic pruning are very active during this developmental period. However, evidence in monkeys also indicates impaired cognitive function at ages 5-8 years with Pb exposure starting after

early infancy ([Rice, 1992b](#); [Rice and Gilbert, 1990a](#); [Rice, 1990](#); [Rice and Gilbert, 1990b](#)). Epidemiologic studies also found cognitive function decrements associated with blood Pb levels measured during various lifestages and time periods. Distinguishing among the effects of Pb exposures at different time periods is difficult in epidemiologic studies due to the high correlations commonly found among blood Pb levels within children over time. Studies of young children ages 6 months to 3 years found lower MDI in association with higher prenatal maternal or cord and postnatal child blood Pb levels; some found larger magnitude decrements for prenatal blood Pb ([Hu et al., 2006](#); [Gomaa et al., 2002](#)). However, in older children, ages 4-17 years, in whom cognitive function is more stable and reliably measured, larger decrements in cognitive function were found in association with increases in postnatal blood Pb levels (concurrent, earlier childhood, and cumulative average) than increases in prenatal blood Pb levels. There was no clear indication of an individual critical lifestage, timing, or duration of Pb exposure associated with cognitive function decrements in children. Because of the contribution of bone Pb levels to concurrent blood Pb levels in children, associations with concurrent blood Pb levels may reflect an effect of past and/or recent Pb exposures.

The consideration for potential confounding varied among studies. Most studies adjusted for SES-related factors such as the Hollingshead Index, household income, and/or parental education. Several, in particular the prospective studies, additionally adjusted for parental cognitive function and caregiving quality mostly evaluated as HOME score. A few studies considered nutritional factors. Not many recent studies considered potential confounding by parental caregiving quality or nutritional factors. Controlling for SES is difficult as it can be highly correlated with Pb exposure and there is no single measure that fully represents SES. Residual confounding also is possible by factors not considered or measured inadequately. The combination of evidence from prospective studies that considered several well-characterized potential confounding factors plus evidence that Pb exposure induces impairments in cognitive function in animals, in particular, for similar constructs as those associated with Pb biomarkers in children, increase confidence that the associations observed between blood Pb levels and cognitive function in children represent a relationship with Pb exposure.

In conclusion, multiple prospective studies conducted in diverse populations consistently demonstrate associations of higher blood and tooth Pb levels with lower FSIQ, executive function, and academic performance and achievement in children. Most studies examined representative populations and had moderate to high follow-up participation without strong indication of selection bias. Associations between blood Pb level and cognitive function decrements were found with adjustment for several potential confounding factors, most commonly, SES, parental IQ, parental education, and parental caregiving quality. Among studies that examined early childhood blood Pb levels (i.e., age < 3

years), considered peak blood Pb levels in their analysis (i.e., peak < 10 µg/dL), or examined concurrent blood Pb levels in young children (i.e., age 4 years), blood Pb-associated decrements in cognitive function were found in populations of children ages 4-11 years with mean or group blood Pb levels in the range of 2 to 8 µg/dL. Neither epidemiologic nor toxicological evidence has identified an individual critical lifestage or time period of Pb exposure within childhood that is associated with cognitive function decrements. Several epidemiologic studies found a supralinear concentration-response relationship. Examination of children with blood Pb levels in the range <1 (at or below detection limits) to 10 µg/dL, with consideration of early or peak childhood blood Pb levels, has not identified a threshold for cognitive function decrements. Evidence in children was clearly supported by observations of Pb-induced impairments in learning, memory, and executive function in rodents and monkeys. Several studies in animals indicated cognitive impairments with prenatal, lactational, and lifetime (with or without prenatal) Pb exposures that resulted in blood Pb levels of 10-25 µg/dL. The mode of action for Pb-associated cognitive function decrements is supported by observations of Pb-induced impairments in neurogenesis, synaptogenesis and synaptic pruning, LTP, and neurotransmitter function in the hippocampus, prefrontal cortex, and nucleus accumbens. The associations consistently found for FSIQ and other measures of cognitive function in prospective studies of children with adjustment for SES, parental education, and parental caregiving quality and the biological plausibility provided by evidence in animals for impairments in learning, memory, and executive function with relevant Pb exposures and evidence describing modes of action are sufficient to conclude that there is a causal relationship between Pb exposure and decrements in cognitive function in children.

4.3.15.2 Evidence for Externalizing Behaviors: Attention, Impulsivity, and Hyperactivity in Children

Attention, impulsivity, and hyperactivity are evaluated together because they are included within the attention deficit hyperactivity disorder domain of externalizing behaviors ([as reviewed in Whitcomb and Merrell, 2012](#)). Although examined less extensively than cognitive function, a causal relationship between Pb exposure and attention decrements, impulsivity, and hyperactivity in children is supported by multiple lines of evidence: 1) findings from prospective studies in diverse populations for associations with blood or tooth Pb levels; 2) coherence with evidence in animals with relevant Pb exposures and; 3) evidence for plausible modes of action ([Table 4-17](#)). As described below, this conclusion is based on evidence primary for attention decrements assessed with neuropsychological testing or rated by parents or teachers and supporting evidence for impulsivity and hyperactivity. The few studies showing blood Pb-associated increases in ADHD prevalence are not a major consideration because of the cross-sectional or case-control

design of studies and inconsistent consideration for potential confounding by factors such as SES, parental education, or parental caregiving quality ([Section 4.3.3.1](#)).

Prospective studies provided key evidence for associations of childhood blood and tooth Pb levels with attention decrements or hyperactivity in children ages 7-17 years and young adults ages 19-20 years. Not all results were statistically significant, but results within and across studies mostly showed a pattern of attention decrements or hyperactivity with higher blood or tooth Pb level. The pattern of association observed within studies increases confidence that the evidence is not unduly biased by the increased probability of finding associations by chance alone. The results from prospective studies better characterized the temporal sequence between Pb exposure and measured behaviors than did cross-sectional studies. Associations were found in populations in the U.S., U.K., Australia, and New Zealand ([Table 4-17](#)). Most studies had population-based recruitment from prenatal clinics, hospitals at birth, or schools and had moderate to high participation. A few prospective studies had greater loss-to-follow-up in certain groups, for example, those with lower SES, earlier FSIQ, or HOME score. This potential selection bias may have reduced the generalizability of findings to the original study population, but there was not a strong indication that participation was biased to those with higher blood Pb levels and greater behavioral decrements.

Key evidence provided by prospective studies supported associations of blood and tooth Pb levels with attention decrements as assessed using neuropsychological tests or parent or teacher ratings using widely-used structured questionnaires ([Section 4.3.3.1](#)). Thus, the collective evidence does not appear to be unduly influenced by biased reporting of such behaviors by parents of children with higher blood or tooth Pb levels. Prospective studies that examined attention with the CPT found associations of higher prenatal or earlier childhood (age 6 year, age 3-60 month) blood Pb levels or tooth (from 1st/2nd grade) Pb levels with increases in commission, omission, or reaction time errors in adolescents ages 15-17 years in Cincinnati ([Ris et al., 2004](#)) and young adults 19-20 years in Chelsea/Somerville, MA ([Bellinger et al., 1994a](#)). Results from prospective studies also indicated associations of lifetime (to ages 11-13 years) average blood Pb levels and tooth (from ages 6-8 years) Pb levels with lower parental and teacher ratings of attention in children ages 8-13 years in Australia, New Zealand, and Boston, MA ([Burns et al., 1999](#); [Fergusson et al., 1993](#); [Leviton et al., 1993](#)). A recent prospective study in the U.K. found that children ages 7-8 years with age 30 month blood Pb levels > 10 µg/dL had higher teacher ratings of hyperactivity but not selective attention compared with children with blood Pb levels 0-2 µg/dL ([Chandramouli et al., 2009](#)). Results from children participating in a chelation trial suggested a blood Pb-hyperactivity relationship that was mediated through an association with IQ ([Chen et al., 2007](#)). Previous findings for hyperactivity were limited to cross-sectional and case-control studies ([Section 4.3.3.1](#)).

Lifetime average blood Pb levels were not associated with ratings of attention in younger children ages 4-5 years in Yugoslavia ([Wasserman et al., 2001](#)). However, attention may be less reliably rated in such young children.

Attention decrements were associated with biomarkers of Pb exposure representing various lifestages and time periods. Prospective studies did not examine a detailed Pb biomarker history, and results do not identify an individual critical lifestage, time period, or duration of Pb exposure associated with attention decrements. Associations in prospective studies for attention decrements with tooth Pb level, early childhood average and lifetime average blood Pb levels point to an effect of cumulative Pb exposure. Prospective studies did not examine biomarkers Pb exposure measured closer to the time of behavioral assessment. Across prospective studies, attention decrements were associated with blood Pb levels that had population means of 6.8 and 8.3 µg/dL for prenatal (maternal or cord) blood, 13.4 for age 3-60 month average blood, and 14 µg/dL for lifetime average blood. In Yugoslavia study with null results, the mean lifetime average blood Pb level was 7.2 µg/dL, similar to those associated with attention decrements in some of the other prospective studies.

A causal relationship between Pb exposure and attention, hyperactivity, and impulsivity is supported further by the consideration for several potential confounding factors in prospective studies. Although the specific factors varied by study, prospective studies adjusted for factors such as SES, parental IQ, maternal education, self drug use, prenatal drug and alcohol exposure, birth outcomes, and parental caregiving quality. Parental caregiving quality has been speculated as a means by which parental psychopathology may influence child Pb exposure. However, a clear relationship between parental psychopathology and poorer parenting behavior has not been established ([as reviewed in Johnston et al., 2012](#)), and adjustment for parental psychopathology may adjust for Pb exposure itself. At present, there is not evidence to support consideration of parental psychopathology as a direct confounding factor, i.e., contributing to a spurious association with Pb biomarkers when there is none. However, if there is a noncausal correlation between parental psychopathology and Pb exposure and it is mediated by parental caregiving quality, then adjustment for parental caregiving quality also may help address potential confounding by parental psychopathology.

Consistent with previous prospective studies, cross-sectional studies found associations of higher concurrent blood or tooth Pb level with attention decrements, impulsivity, and hyperactivity in children ages 3-16 years ([Table 4-17](#)). Because of the greater uncertainty about the potential for reverse causation, cross-sectional results are a lesser consideration in conclusions. The cross-sectional studies included a mix of previous and recent studies, a mix of preschool-aged and school-aged children, and a wide range of population mean

blood Pb levels (1-12 µg/dL). Among the studies examining populations in the lower part of this blood Pb distribution (means: 5-6.5 µg/dL for ages 4-7.5 years) and with more extensive consideration for potential confounding, most found associations with attention decrements, hyperactivity, and/or impulsivity ([Plusquellec et al., 2010](#); [Chiodo et al., 2007](#); [Chiodo et al., 2004](#)). All of these studies adjusted for or considered potential confounding by SES as well as parental IQ, education, and caregiving quality. Among children ages 4-6 years in whom behavior may be less reliably measured, concurrent blood Pb level was associated with higher examiner ratings of attention decrements, impulsivity, and hyperactivity in Inuit children ages 5-6 years in Canada ([Plusquellec et al., 2010](#)) but not in the Rochester cohort at age 4.5 years ([Canfield et al., 2003b](#)). Also, in the Rochester cohort, the association with attention was attenuated only after adjustment for color/shape knowledge and child IQ. These inter-related outcomes may share some variance. Among older children (age 7-7.5 years) in Detroit, MI, higher concurrent blood Pb level (means: 5; 5.4 µg/dL) was associated with higher examiner or teacher ratings of attention decrements and hyperactivity ([Chiodo et al., 2007](#); [Chiodo et al., 2004](#)). These studies also found associations with attention decrements as assessed by the CPT, although not strongly with all examined indices of the CPT. These populations had high prevalence of prenatal alcohol or drug exposure, but results did not indicate confounding by these other exposures. Nonetheless, the presence of these other exposures may limit the generalizability of results. While higher-quality cross-sectional evidence in populations with mean blood Pb levels 5-6.5 µg/dL show associations of blood Pb level with attention decrements, impulsivity, and hyperactivity, there are limitations related to young age of subjects, generalizability of the study population, or potential contribution of higher blood Pb levels earlier in childhood.

Other cross-sectional studies examined even lower concurrent blood Pb levels (means: 1-3.7 µg/dL) and found associations with attention decrements, impulsivity, hyperactivity, or a composite index of ADHD-related behaviors in children ages 8-17 years ([Cho et al., 2010](#); [Nicolescu et al., 2010](#); [Froehlich et al., 2009](#); [Nigg et al., 2008](#)). These associations were found with parental or teacher ratings, with attention measured by the CPT, and impulsivity measured with response inhibition tests. However, results have weaker implications because of less extensive consideration for potential confounding, case-control study design, and uncertainty regarding the influence of higher blood Pb levels earlier in childhood. Studies varied in adjusting results for SES and parental education, and none considered parental caregiving quality. Most studies of populations with relatively high concurrent blood Pb levels (means 11-12 µg/dL for ages 3-11 years) also found that higher blood Pb levels were associated with higher parent or teacher ratings of attention decrements and hyperactivity ([Roy et al., 2009](#); [Kordas et al., 2007](#); [Silva et al., 1988](#)). Most cross-sectional studies of higher blood Pb levels found associations with adjustment for SES, child age, sex, family structure, and parental

education, but most did not examine parental caregiving quality. With the exception of findings from the Rochester cohort ([Canfield et al., 2003b](#)), studies generally found associations between blood Pb level and attention decrements, impulsivity, and hyperactivity with adjustment for child IQ or other measure of cognitive function ([Cho et al., 2010](#); [Chandramouli et al., 2009](#); [Nigg et al., 2008](#); [Silva et al., 1988](#)). These findings add support for higher Pb exposures having effects on attention, impulsivity, and hyperactivity, independent of effects on cognitive function.

Further support for a causal relationship between Pb exposure and attention, impulsivity, and hyperactivity is provided by observations of greater impulsivity in animals with relevant dietary Pb exposures. Evidence in rodents and monkeys demonstrates Pb-induced impaired response inhibition by decreased interresponse times and increased overall response rates in FI reinforcement schedules ([Table 4-7](#)) ([Rossi-George et al., 2011](#)), slower acquisition of a DRL schedule ([Rice and Gilbert, 1985](#)), and response perseveration on tests of discrimination reversal learning and FR/waiting for reward ([Brockel and Cory-Slechta, 1999b, 1998](#); [Gilbert and Rice, 1987](#)). In particular, coherence is found with observations in children of associations between blood Pb levels and poorer performance on the stop signal task, which also measures response inhibition. Impulsivity in rodents and monkeys was found with gestational, post-weaning and lifetime (from gestation or birth) dietary Pb exposures that resulted in blood Pb levels 11-29 µg/dL ([Table 4-17](#) and [Section 4.3.3.1](#)). In animals, the effects of Pb exposure on sustained attention were inconsistent as assessed with signal detection or discrimination reversal tests with distracting stimuli ([Table 4-17](#) and [Section 4.3.3.1](#)). A recent study did not clearly indicate an effect of Pb on activity in male mice as an increase was found only with amphetamine co-treatment ([Leasure et al., 2008](#)); thus, the findings may not be directly comparable to observations of Pb-associated increases in hyperactivity in children.

Additional support for a causal relationship between Pb exposure and attention decrements, impulsivity, and hyperactivity is provided by evidence describing modes of action. These behaviors have been linked with changes in the prefrontal cerebral cortex, cerebellum, and hippocampus, and Pb exposure has been found to affect development and neuronal processes in these regions. For example, Pb has been found to affect dopaminergic neurons of the frontal cortex and striatum of the brain by altering dopamine release and receptor density. Other lines of evidence supporting the mode of action for the effects of Pb exposure on attention, impulsivity, and hyperactivity include Pb-induced changes in neurogenesis, synapse formation, and synaptic plasticity.

In conclusion, although examined less extensively than cognitive function, several prospective studies demonstrated associations of blood or tooth Pb levels measured years

before outcomes with attention decrements and hyperactivity in children 7-17 years and young adults ages 19-20 years as assessed using objective neuropsychological tests and rated by parents and teachers. Most of these prospective studies examined representative populations without indication of participation conditional on blood Pb levels and behavior. The results from prospective studies were adjusted for potential confounding by SES as well as parental education and caregiving quality, with some studies also considering parental cognitive function, birth outcomes, substance abuse, and nutritional factors. In prospective studies, blood Pb-associated attention decrements and hyperactivity were found in populations with prenatal (maternal or cord), age 3-60 month average, age 6 year, or lifetime average (to age 11-13 years) mean blood Pb levels of 7 to 14 µg/dL and in groups with age 30 month blood Pb levels >10 µg/dL. Most well-conducted cross-sectional studies that examined several potential confounding factors found associations of attention decrements, impulsivity, and hyperactivity in children ages 5-7.5 years with concurrent blood Pb levels with means of 5-5.4 µg/dL but cannot establish temporality or exclude the influence of higher blood Pb levels earlier in childhood. Biological plausibility for observations in children is provided by several findings in animals for increases in impulsivity or impaired response inhibition with relevant post-weaning and lifetime Pb exposures that resulted in blood Pb levels of 11 to 29 µg/dL. The mode of action for Pb-associated attention decrements, impulsivity, and hyperactivity is supported by observations of Pb-induced impairments in neurogenesis, synaptic pruning, and dopamine transmission in the prefrontal cerebral cortex, cerebellum, and hippocampus. The consistency of epidemiologic evidence for attention decrements and hyperactivity from prospective studies and the biological plausibility provided by evidence for Pb-induced impulsivity in animals and for underlying modes of action is sufficient to conclude that there is a causal relationship between Pb exposure and effects on attention, impulsivity, and hyperactivity in children.

4.3.15.3 Evidence for Externalizing Behaviors: Conduct Disorders in Children and Young Adults

There are two domains of conduct disorders: undersocialized aggressive conduct disorder and socialized aggressive conduct disorder ([as reviewed in Whitcomb and Merrell, 2012](#)). In this evaluation of a relationship with Pb exposure, the two domains are combined because it is difficult to differentiate between these two domains in the available epidemiologic studies. Criminal offenses are included in the evaluation because they can be predicted by earlier conduct disorders ([Soderstrom et al., 2004](#); [Babinski et al., 1999](#); [Pajer, 1998](#)). A causal relationship is likely to exist between Pb exposure and conduct disorders in children and young adults based primarily on epidemiologic evidence ([Section 4.3.3.2](#)). Key evidence is provided by most previous and recent prospective

studies that found associations of blood Pb levels with higher parent and teacher ratings of delinquent, aggression, and antisocial behavior in children ages 8-17 years from diverse locations and SES (i.e., U.K., Cincinnati, Port Pirie, Australia) ([Table 4-12](#) and [Table 4-17](#)). Associations were found with lifetime average blood Pb levels in boys ages 11-13 years in Port Pirie, Australia ([Burns et al., 1999](#)), with age 30 month blood Pb levels in children ages 8 years in the U.K. ([Chandramouli et al., 2009](#)), and age 0-6 year average blood Pb levels in the Cincinnati cohort at age 15-17 years ([Dietrich et al., 2001](#)). Additional support was provided recent prospective studies finding associations of higher earlier childhood blood (age 6 years) or tooth (from ages 6-8 years) Pb levels with criminal offenses in young adults in Cincinnati and Christchurch, New Zealand, ages 19-24 years, as assessed through government records ([Fergusson et al., 2008](#); [Wright et al., 2008](#)). In the Cincinnati cohort, a 1 µg/dL increase in age 6 year blood Pb level was associated with an increased risk of criminal arrests at age 19-24 years with an RR of 1.05 (95% CI: 1.01, 1.09). The moderate to high follow-up participation and associations found with parent and teacher ratings of behaviors related to conduct disorders do not provide strong evidence for biased participation or reporting of behaviors for children with higher blood Pb levels. Studies of criminal offenses and ratings of behaviors related to conduct disorders found associations with adjustment for several potential confounding factors such as SES, exposure to smoking, drugs, or alcohol, and parental caregiving quality. Adjustment for parental caregiving quality may also address potential confounding by parental psychopathology. Parental caregiving quality has been speculated as a means by which parental psychopathology may influence child Pb exposure. However, a clear relationship between parental psychopathology and poorer parenting behavior has not been established ([as reviewed in Johnston et al., 2012](#)). There is not evidence to support consideration of parental psychopathology as a direct confounding factor.

Supporting evidence was provided by cross-sectional studies, including the large analysis of 2,867 children ages 8-15 years participating in NHANES, which found that compared with children with concurrent blood Pb levels 0.2-0.7 µg/dL (detection limit=0.3 µg/dL), children with concurrent blood Pb levels 0.8-1.0 µg/dL had higher odds of conduct disorder as assessed by parents with adjustment for age, sex, race, poverty income ratio, and smoking exposure ([Braun et al., 2008](#)). Chiodo et al. (2007) found an association between concurrent blood Pb level and teacher ratings of social problems and delinquent behavior in children ages 7 years in Detroit, MI after extensive consideration for potential confounding factors including parental caregiving quality as well as prenatal drug exposure, which was prevalent in the population. Further supporting the consistency of association between blood Pb levels and behaviors related to conduct disorders in children, a recent meta-analysis found that evidence was robust to heterogeneity in study

design, definition and assessment method of conduct disorders, potential confounding variables examined, and range of blood Pb levels ([Marcus et al., 2010](#)).

Associations of measures of conduct disorders in children and young adults with earlier childhood, earlier childhood average, and lifetime average blood Pb levels, tooth Pb levels, and concurrent bone Pb levels point to the effects of early childhood or cumulative Pb exposures. Most prospective studies did not analyze Pb biomarker levels at multiple lifestages or time periods and thus did not provide information on potential associations with more recent blood Pb levels or differences in association among Pb biomarkers at various time periods. With respect to blood Pb levels, an association with criminal offenses was found in young adults ages 19-24 years with a mean age 6 year blood Pb level of 6.8 µg/dL, and associations with behaviors related to conduct disorders were found in children age 8 years with age 30 month blood Pb levels >10 µg/dL, boys ages 11-13 years with a mean lifetime average blood Pb level of 14 µg/dL, and children ages 15-17 years with a median age 6 year blood Pb level of 12 µg/dL ([Table 4-17](#)). Cross-sectional studies found associations with concurrent blood Pb level that were lower: 0-8-1.0 µg/dL for ages 8-15 years and a mean of 5 µg/dL for children age 7 years. However, the influence by higher past Pb exposures cannot be excluded.

Despite evidence from several high-quality epidemiologic studies that have examined several potentially important confounding factors, an important uncertainty remains. Evidence of Pb-induced aggression in animals was inconsistent, with increases in aggression found in some studies of adult animals with gestational plus lifetime Pb exposure but not juvenile animals ([Section 4.3.3.2](#)). The lack of biological plausibility from animal evidence and the relatively small body of epidemiologic evidence produces uncertainty regarding the effects of Pb exposure independent from other factors.

In conclusion, the few prospective studies consistently indicate that earlier childhood (age 30 months, 6 years) or lifetime average (to age 11-13 years) blood Pb levels or tooth (from ages 6-8 years) Pb levels are associated with criminal offenses in young adults ages 19-24 years and with higher parent and teacher ratings of behaviors related to conduct disorders in children ages 8-17 years. Pb-associated increases in conduct disorders were indicated in populations with mean blood Pb levels 7-14 µg/dL. Supporting evidence is provided by a few cross-sectional studies and a meta-analysis of prospective and cross-sectional studies. Associations with lower blood Pb levels, as found in the cross-sectional studies, may have been influenced by higher earlier Pb exposures. Overall, associations were found without indication of strong selection bias and with adjustment for SES, family functioning, parental education, IQ, and caregiving quality, and maternal smoking and substance abuse. Evidence for Pb-induced aggression in animals is mixed. The consistent epidemiologic evidence from prospective and cross-sectional studies for

criminal offenses and measures of conduct disorders, but uncertainty due to lack of clear evidence for aggression in animals, is sufficient to conclude that a causal relationship is likely to exist between Pb exposure and conduct disorders in children and young adults.

4.3.15.4 Evidence for Internalizing Behaviors in Children

A causal relationship is likely to exist between Pb exposure in children and internalizing behaviors, including withdrawn behavior and symptoms of depression, fearfulness, and anxiety based on evidence from a few epidemiologic studies and a few animal toxicological studies. Internalizing behaviors have been examined to a lesser extent than cognitive function or externalizing behaviors. However, supporting evidence is provided by a few previous prospective studies that found associations of higher lifetime average blood or tooth Pb levels with higher parent and teacher ratings of withdrawn behavior or symptoms of anxiety and depression in school-aged children, 8-13 years ([Burns et al., 1999](#); [Bellinger et al., 1994b](#)) (Section 4.3.4.1, Table 4-17). These prospective studies followed children from birth and had moderate follow-up participation to later childhood. Participation was not conditional on early childhood blood Pb levels, and associations were found with both parent and teacher ratings of internalizing behaviors, reducing the likelihood of undue influence of biased participation and behavioral ratings by parents of children with higher Pb exposures. Internalizing behaviors were assessed with widely-used structured questionnaires such as the Child Behavior Checklist but not assessed as clinically-diagnosed conditions such as depression.

The analysis of the Port Pirie, Australia cohort had the most extensive consideration of potential confounding. Among only the 163 girls, ages 11-13 years, Burns et al. ([1999](#)) found that a 1 µg/dL increase in lifetime average blood Pb level in the interval 13.3-14.9 µg/dL (10th-90th percentiles) was associated with an increased odds of an anxious/depressed parental rating above the median of 1.07 (95% CI: 1.01, 1.14) with the adjustment for several SES-related variables and factors related to parental caregiving including HOME score, family functioning score, and current maternal psychopathology. In a Boston-area cohort, Pb level in deciduous teeth (from age 6 years) was associated with a higher teacher rating of a composite of anxious and social withdrawn behaviors in children ages 8 years with adjustment for receiving public assistance at birth and maternal education ([Bellinger et al., 1994b](#)). Tooth Pb level was not associated with internalizing behaviors in another Boston-area cohort at age 19-20 years ([Bellinger et al., 1994a](#)). In the Yugoslavia cohort, higher lifetime average blood Pb levels (mean: 7.2 µg/dL) were associated with higher maternal ratings of anxious-depressed and withdrawn behaviors in 191 children ages 4-5 years, with stronger associations found with delinquent behaviors ([Wasserman et al., 2001](#)). Behavior ratings may be less reliable in

these younger children. With respect to critical lifestages and durations of Pb exposure, evidence from prospective studies for associations with tooth Pb levels and lifetime average blood Pb levels indicates an effect of cumulative Pb exposure on increasing internalizing behaviors in children. While these studies adjusted for SES, birth outcomes, and parental education, few considered parental caregiving quality. Thus, there is uncertainty regarding potential confounding by parental caregiving quality.

Cross-sectional studies, including several recent studies, indicated associations between concurrent blood Pb levels and internalizing behaviors in children ages 3-16 years. Among the cross-sectional studies with more extensive consideration for potential confounding and examination of relatively low concurrent blood Pb levels, i.e., means ~ 5 $\mu\text{g/dL}$, results were inconsistent ([Table 4-17](#)). Previously, Chiodo et al. ([2004](#)) found that among children age 7 years in Detroit, MI, HOME score, SES, maternal education, and prenatal alcohol and drug exposure did not influence associations between blood Pb level (mean: 5 $\mu\text{g/dL}$) and internalizing behaviors. While the high prevalence of prenatal alcohol and drug exposure may not affect internal validity of results, they may limit the generalizability of findings. A lack of association between concurrent blood Pb level and internalizing behaviors was reported in Inuit children age 5 years in Quebec, Canada with a mean blood Pb level of 5.4 $\mu\text{g/dL}$ with consideration of potential confounding by parental caregiving quality, caregiver education and IQ, blood Hg and PCB levels, and prenatal smoking and alcohol exposure ([Plusquellec et al., 2010](#)).

Some of the uncertainty in the small epidemiologic evidence base regarding a relationship between Pb exposure and internalizing behaviors is addressed by the biological plausibility provided by the coherence with evidence from a few studies that relevant Pb exposures resulted in depression-like behavior or emotionality in rodents, i.e., dietary lactational Pb exposure producing blood Pb levels of 13 and 17 $\mu\text{g/dL}$ ([Beaudin et al., 2007](#); [Dyatlov and Lawrence, 2002](#)). Other studies found Pb-induced increases in emotionality, depression-like behavior, and tactile defensiveness in animals with blood Pb levels >30 $\mu\text{g/dL}$ after gestational/lactational, lactational, or other early postnatal Pb exposure ([Section 4.3.4.2](#)). Biological plausibility for Pb-associated increases in internalizing behaviors also is provided by evidence that describes mode of action, including Pb-induced changes in the HPA axis ([Section 4.3.2.3](#)) and dopaminergic and GABAergic systems (([Sections 4.2.2.2](#), [4.3.10.4](#), and [4.3.10.8](#)), which are found to affect mood and emotional state.

In conclusion, prospective studies in a few populations demonstrate associations of higher lifetime average blood (mean: ~ 14 $\mu\text{g/dL}$, age 11-13 years) or childhood tooth (from ages 6-8 years) Pb levels with higher parent and teacher ratings of internalizing behaviors such as withdrawn behavior and symptoms of depression, fearfulness, and

anxiety in children ages 8-13 years. The lack of selective participation by blood Pb level and associations found with parental and teacher behavioral ratings do not provide strong indication of selection bias. The few cross-sectional studies in populations ages 5 and 7 years with mean concurrent blood Pb levels of 5 µg/dL produced inconsistent findings. Pb-associated increases in internalizing behaviors were found with adjustment for maternal education and SES-related variables, and in a few studies, consideration for parental caregiving quality. Some uncertainty in the epidemiologic evidence for the effects of Pb on internalizing behaviors is addressed by the biological plausibility provided by a few findings in animals with dietary lactational Pb exposure producing blood Pb levels relevant to this ISA. Additional toxicological evidence supports modes of action, including Pb-induced changes in the HPA axis and dopaminergic and GABAergic systems. The evidence from prospective studies in a few populations of children and the coherence with evidence from a few animal studies with relevant Pb exposures and with evidence for mode of action but some uncertainty related to potential confounding by parental caregiving quality in studies of children is sufficient to conclude that a causal relationship is likely to exist between Pb exposure and internalizing behaviors in children.

4.3.15.5 Evidence for Auditory Function Decrements in Children

A causal relationship is likely to exist between Pb exposure and auditory function decrements in children based primarily on epidemiologic evidence provided by a previous prospective study indicating associations of poorer auditory processing with infancy, childhood, and lifetime average blood Pb levels in 215 children age 5 years ([Dietrich et al., 1992](#)) and previous large (n = >3,000) cross-sectional NHANES and HHANES studies showing associations of increasing hearing thresholds with concurrent blood Pb levels in children ages 4-19 years ([Schwartz and Otto, 1991, 1987](#)). The high follow-up participation from birth in the Cincinnati cohort and the examination of multiple exposures and outcomes in NHANES and HHANES reduce the likelihood of biased participation by children specifically with high blood Pb levels.

The epidemiologic evidence in children is strengthened by the consideration for several potential confounding factors. In the Cincinnati cohort at age 5 years, higher prenatal maternal, neonatal (age 10 day, mean: 4.8 µg/dL), and various postnatal blood Pb levels were associated with poorer auditory processing with adjustment for SES, HOME score, several birth outcomes, and maternal alcohol consumption and with consideration for factors such as maternal smoking and child health ([Dietrich et al., 1992](#)). In NHANES and HHANES, higher concurrent blood Pb levels (median: 8 µg/dL) were associated with

increased hearing thresholds with adjustment for age, sex, race, family income, parental education, and nutritional factors ([Schwartz and Otto, 1991](#), [1987](#)).

Additional support for a relationship between Pb exposure and auditory function decrements in children is provided by evidence supporting modes of action. A previous prospective study in children in Mexico City (n = 100-113) found associations of prenatal maternal (range: 1-8 µg/dL) and age 1 and 4 year blood Pb levels (age 2 year mean: 10.8 µg/dL) with lower auditory evoked potentials ([Rothenberg et al., 2000](#)). Increased hearing thresholds or latencies in brainstem auditory evoked potentials were found in adult monkeys (ages 8-13 years) with lifetime Pb exposure beginning at gestation or birth ([Rice, 1997](#); [Lilienthal and Winneke, 1996](#)). Smaller effects were induced by Pb exposure during gestation only or lactation only (to age 5 months) ([Laughlin et al., 2009](#)). In animals, auditory effects were examined with higher Pb exposures than those relevant to this ISA (i.e., blood Pb levels 33-150 µg/dL at peak or testing); thus, it is difficult to assess coherence with observations in children.

In conclusion, evidence from a prospective study and cross-sectional studies in a few U.S. populations of children indicates associations of higher blood Pb level with increases in hearing thresholds and decreases auditory processing or auditory evoked potentials with adjustment for potential confounding by SES in most studies and by child health and nutritional factors in some studies. The high participation rates, particularly in the prospective study with follow-up from birth, reduce the likelihood of biased participation by children with higher blood Pb levels. Across studies, associations were found with blood Pb levels measured at various time periods, including prenatal maternal, neonatal (10 day, mean 4.8 µg/dL), lifetime average (to age 5 years), and concurrent (ages 4-19 years) blood Pb levels (median 8 µg/dL). Evidence for Pb-associated increases in hearing thresholds or latencies of auditory evoked potentials was found in adult monkeys with lifetime dietary Pb exposure. However, these effects in adult animals were found with higher peak or concurrent blood Pb levels (i.e., 33-150 µg/dL) than those relevant to this ISA; thus, the biological plausibility for epidemiologic observations is unclear. The evidence in children, particularly that from a prospective study, but uncertainties related to effects on auditory function in juvenile animals with relevant Pb exposures, is sufficient to conclude that a causal relationship is likely to exist between Pb exposure and decrements in auditory function in children.

4.3.15.6 Evidence for Visual Function Decrements in Children

The effects of relevant Pb exposures on visual function related to CNS changes specifically in children have been examined in few toxicological and epidemiologic studies. An epidemiologic study in children ([Rothenberg et al., 2002b](#)) and toxicological studies in adult rats found Pb-associated supernormal ERGs ([Fox et al., 2008](#)). However, the relevance of supernormal ERGs to visual function is unclear. Adult animal studies also showed subnormal ERGs depending on the timing of Pb exposure and blood Pb level. Toxicological studies also have characterized potential mechanisms leading to retinal changes including alterations in morphology and cell architecture, signaling, ATPase activity, neurotransmitter levels, cell proliferation, and retinal cell apoptosis. A study of infant monkeys did not find infancy Pb exposure to affect on visual acuity ([Laughlin et al., 2008](#)) and used higher exposures than those relevant to this ISA (blood Pb levels 35-40 µg/dL). Because the available epidemiologic and toxicological evidence is of insufficient quantity, quality, and consistency, the evidence is inadequate to determine that a causal relationship exists between Pb exposure and visual function in children.

4.3.15.7 Evidence for Motor Function Decrements in Children

A causal relationship is likely to exist between Pb exposure and motor function decrements in children based mostly on evidence from previous prospective epidemiologic studies with some supporting toxicological evidence. In the Cincinnati cohort, higher neonatal, concurrent, and lifetime average blood Pb levels (means: 4.8, 10, and 12 µg/dL, respectively) were associated with poorer fine and gross motor function in children at age 6 years ([Bhattacharya et al., 1995](#); [Dietrich et al., 1993a](#)), and higher age 0-5 year average (mean: 12 µg/dL) and 78 month blood Pb levels were associated with poorer fine and gross motor function in children at ages 15-17 years ([Bhattacharya et al., 2006](#); [Ris et al., 2004](#)). In the Yugoslavian cohort, higher lifetime average blood Pb level was associated with decrements in fine but not gross motor function at age 4.5 years ([Wasserman et al., 2000b](#)). In these studies, follow-up participation was not conditional on blood Pb levels and motor function, reducing the likelihood of selection bias affecting results. Motor function was assessed using varied but widely-used, structured tests. The evidence from the Cincinnati and Yugoslavia cohorts is substantiated by the consideration of several potential confounding factors such as SES, parental caregiving quality, child health, and in adolescents, marijuana use ([Ris et al., 2004](#)). In the prospective studies, the population mean blood Pb levels were higher than those of most of the current U.S. population. Recent cross-sectional studies examining concurrent blood Pb levels that were lower, means 2-5 µg/dL, produced mixed results. Higher concurrent

blood Pb level was associated with poorer motor function with adjustment for several potential confounding factors in 110 Inuit children ages 4-6 years living in subsistence fishing communities ([Despres et al., 2005](#)) but not in a more representative population of 534 children ages 6-10 years in New England ([Surkan et al., 2007](#)) or in children ages 7-16 years in Korea ([Min et al., 2007](#)).

Epidemiologic evidence is supported by observations of poorer balance as assessed with the rotarod test in male (not female) mice with relevant blood Pb levels, i.e., 10 µg/dL after dietary Pb exposure from gestation to PND10 ([Leasure et al., 2008](#)). Other toxicological studies produced mixed results for effects on endurance, coordination, and balance ([Section 4.3.7](#)) but are less relevant to this ISA because of the higher Pb exposure concentrations examined, i.e., those producing blood Pb levels >30 µg/dL.

In conclusion, evidence from prospective studies in a few populations indicates associations of decrements in fine and gross motor function with higher neonatal, concurrent, and lifetime average blood Pb levels in children ages 4.5-6 years and with higher earlier childhood (ages 0-5 year average, age 78 months) blood Pb levels in children ages 15-17 years. The means for these blood Pb metrics ranged from 4.8 to 12 µg/dL. These associations were found with adjustment for several potential confounding factors, including SES, parental caregiving quality, and child health and without indication of substantial selection bias. Evidence from cross-sectional studies was less consistent. The biological plausibility for associations observed in children is provided by a study that found poorer balance in male mice with relevant gestational to early postnatal (PND10) Pb exposures. The evidence in children, particularly from the few prospective studies, and the coherence with limited available findings in mice is sufficient to conclude that a causal relationship is likely to exist between Pb exposure and decrements in motor function in children.

4.3.15.8 Evidence for Cognitive Function Decrements in Adults

A causal relationship is likely to exist between Pb exposure and cognitive function decrements in adults based on prospective and cross-sectional epidemiologic studies that indicate associations with bone Pb level and coherence with evidence in animals ([Table 4-17](#)). Key evidence for bone Pb levels was provided by recent prospective analyses of the BMS and NAS, with support provided by the cross-sectional Nurses' Health Study. The multiple risk factors and health outcomes examined in these studies reduces the likelihood of biased participation and/or follow-up by adults specifically with higher Pb exposure and lower cognitive function. While the NAS and Nurses' Health Study examined primarily white men and white women, respectively, the BMS examined a

more ethnically diverse population of men and women, increasing the generalizability of findings. There was variability in associations across the various domains of cognitive function tested within studies; however, bone Pb levels were associated with decrements in most of the neuropsychological tests performed. In many studies, bone Pb levels were associated with poorer executive function, visuospatial skills, learning, and memory.

Recent evidence from prospective analyses of the NAS and BMS cohorts expanded upon previous cross-sectional evidence by improving characterization of the temporal sequence between Pb exposure and cognitive function declines in adults (n = 358-965, mean ages 59-69 years) by demonstrating that higher tibia (means: 19, 20 µg/g) or patella (mean: 25 µg/g) bone Pb levels measured at baseline were associated with subsequent declines in cognitive function over 2- to 4-year periods ([Bandeem-Roche et al., 2009](#); [Weisskopf et al., 2007b](#)). The specific potential confounding factors considered differed between studies; both studies adjusted for age and education. Additional adjustment was made for household wealth in the BMS and current alcohol use and current smoking in the NAS.

Evidence from most cross-sectional analyses supported associations between higher bone Pb level and decrements in cognitive function in adults. A strength of cross-sectional studies overall was the adjustment for many of the potential confounding factors described above and also dietary factors, physical activity, medication use, and comorbid conditions ([Rajan et al., 2008](#); [Weuve et al., 2006](#)). Cross-sectional studies generally demonstrated larger decrements in cognitive function in adults per unit increase in tibia or patella Pb levels than concurrent blood Pb levels. Results from the NAS and Nurses' Health Study did not clearly indicate a difference in association with cognitive performance between tibia and patella Pb levels ([Weuve et al., 2009](#); [Weisskopf et al., 2007b](#)). In NHANES analyses, higher concurrent blood Pb levels were associated with lower cognitive function in particular age and genetic variant subgroups but not consistently across the various cognitive tests conducted ([Krieg et al., 2010](#); [Krieg and Butler, 2009](#); [Krieg et al., 2009](#)). NHANES did not have bone Pb measures for comparison.

Because bone Pb is a major contributor to blood Pb levels, blood Pb level also can reflect longer term exposures, including higher past exposures, especially in adults without occupational exposures ([Sections 3.3](#) and [3.7.3](#)). Thus, in the NHANES results, it is difficult to characterize the relative contributions of recent and past Pb exposures to the associations observed between concurrent blood Pb level and cognitive function. The discrepant findings for blood and bone Pb levels in other studies indicate that cumulative Pb exposure that likely included higher past exposures may be a better predictor of cognitive function in adults than is concurrent blood Pb level. Additional support for the

effects of cumulative or past Pb exposure is provided by analyses of a few child cohorts as adults, which indicate that childhood tooth (from 1st/2nd grade) and blood (average to age 10 years) Pb levels are associated with decrements in cognitive function in adults ages 19-30 years ([Mazumdar et al., 2011](#); [Bellinger et al., 1994a](#)). An uncertainty in the evidence for bone Pb levels is potential residual confounding by age. Increasing age is highly correlated with increasing bone Pb level ([Section 3.3.5.2](#)), and distinguishing Pb-related declines in cognitive function from age-related declines with model adjustment is difficult. One explanation for the more variable findings in adults than in children may be that cognitive reserve may compensate for the effects of Pb exposure on learning new information. Among current smelter workers, blood Pb-associated decrements in cognitive function were larger among workers with lower cognitive reserve ([Bleecker et al., 2007a](#)). Compensatory mechanisms may be overwhelmed with age and with higher long-term or cumulative Pb exposure represented by higher bone Pb levels.

Higher blood and bone Pb levels were associated with cognitive function decrements in adults with current or former occupational Pb exposures ([Table 4-17](#)). Some studies examined current workers with a mean concurrent blood Pb level of 31 µg/dL ([Dorsey et al., 2006](#); [Stewart et al., 2002](#)). Among adults with current occupational Pb exposures, both concurrent and cumulative exposures may affect cognitive function. Several of these studies considered potential confounding by a similar set of factors as did studies of adults without occupational Pb exposures but did not examine other occupational exposures such as manganese. In the prospective study of adults with former occupational Pb exposure, peak tibia Pb levels were associated more strongly with cognitive performance than were concurrent blood Pb levels ([Khalil et al., 2009a](#)). Thus, in the absence of higher current Pb exposures, cumulative Pb exposures may have a greater effect on cognitive function in adults.

Additional support for a relationship between Pb exposure and cognitive function decrements in adults is provided by the coherence with evidence in adult animals that lifetime Pb exposure starting from gestation, birth, or after weaning induces learning impairments ([Table 4-17](#) and [Section 4.3.2.3](#)). Biological plausibility also is provided by evidence describing the effects of Pb on modes of action underlying cognitive function. Cognitive function is mediated by actions of the neurotransmitters dopamine and glutamate in the hippocampus, prefrontal cortex, and nucleus accumbens. Experimental studies have shown that Pb induces changes in neurotransmitter release in these regions. Studies also have shown Pb-induced decreases in the magnitude of LTP, which is a major cellular mechanism underlying learning and memory.

In conclusion, in adults without occupational exposure, recent prospective studies in the NAS and BMS cohorts indicate associations of higher baseline tibia (means 19, 20 µg/g) or patella (mean 25 µg/g) Pb levels with declines in cognitive function in adults (>age 50 years) over 2- to 4-year periods. While the specific covariates differed between studies, these bone Pb-associated cognitive function decrements were found with adjustment for potential confounding factors such as age, education, SES, current alcohol use, and current smoking. Supporting evidence is provided by cross-sectional analyses of the NAS, BMS, and the Nurses' Health Study, which found stronger associations with bone Pb level than concurrent blood Pb level. Cross-sectional studies also considered more potential confounding factors, including dietary factors, physical activity, medication use, and comorbid conditions. The multiple exposures and health outcomes examined in many studies reduces the likelihood of biased participation by adults specifically with higher Pb exposure and lower cognitive function. The collective evidence indicates associations in cohorts of white men and women and a cohort of more ethnically diverse men and women. The specific timing, frequency, duration, and magnitude of Pb exposures contributing to the associations observed with bone Pb levels are uncertain. Also uncertain is the potential for residual confounding by age. The effects of recent Pb exposures on cognitive function decrements were indicated in Pb-exposed workers by associations found with blood Pb levels, although these studies did not consider potential confounding by other workplace exposures. The biological plausibility for the effects of Pb exposure on cognitive function decrements in adults is provided by findings that lifetime Pb exposures from gestation, birth, or after weaning induce learning impairments in adult animals and by evidence for the effects of Pb on altering neurotransmitter function in the hippocampus, prefrontal cortex, and nucleus accumbens. The associations between bone Pb level and cognitive function decrements consistently found in the few prospective and cross-sectional studies of adults without occupational Pb exposure, the coherence with animal findings, and toxicological evidence supporting modes of action, but uncertainties related to potential residual confounding by age in epidemiologic studies, are sufficient to conclude that a causal relationship is likely to exist between long-term cumulative Pb exposure and cognitive function decrements in adults.

4.3.15.9 Evidence for Psychopathological Effects in Adults

Evidence indicates that a causal relationship is likely to exist between Pb exposure and psychopathological effects in adults, based on the associations found between concurrent blood or bone Pb level and self-reported symptoms of depression, anxiety, and panic disorder in large cross-sectional studies of adults (i.e., NHANES, NAS) and some supporting toxicological evidence. Higher cord blood δ-ALA level was associated with

schizophrenia in adults in California ([Opler et al., 2008](#); [2004](#)), but because of the lack of assessment of blood or bone Pb levels, results were not a major consideration in conclusions. In both NHANES and NAS, the high participation rates and examination of multiple exposures and outcomes reduces the likelihood that findings are influenced by biased participation or reporting of symptoms by subjects specifically with higher Pb exposures. Symptoms of depression and anxiety were assessed with widely-used structured questionnaires such as the Profile of Mood States, but there is uncertainty regarding the effects of Pb exposure on clinically-diagnosed disorders.

Epidemiologic evidence for associations between blood or bone Pb level and psychopathological effects is strengthened by the adjustment for several potential confounding factors including age, education, smoking, alcohol use, employment status, and SES. Among adults ages 20-39 years participating in NHANES, 369 adults with concurrent blood Pb level ≥ 2.1 $\mu\text{g/dL}$ had the highest OR for self-reported major depressive disorder (OR: 2.3 [95% CI: 1.1, 4.8]) and panic disorder (OR: 4.9 [95% CI: 1.3, 18]) compared with the 449 adults with blood Pb levels 0.3-0.7 $\mu\text{g/dL}$ ([Bouchard et al., 2009](#)). Among NAS men ages 48-94 years, higher concurrent blood, patella, and tibia Pb levels were associated with self-reported anxiety (nervousness, tension), depression, and phobic anxiety (a subcategory of anxiety but defined specifically as persistent fear, irrational and disproportionate to a stimulus) ([Rajan et al., 2007](#); [Rhodes et al., 2003](#)). A 14 $\mu\text{g/g}$ increase in tibia Pb level was associated with a self-reported anxiety score one standard deviation above the mean with an OR of 1.2 (95% CI: 0.98, 1.4) ([Rajan et al., 2007](#)). Because of the cross-sectional design of studies, the temporal sequence between Pb exposure and psychopathological symptoms in adults is uncertain. This uncertainty is somewhat reduced with results for tibia Pb, since it is an indicator of cumulative Pb exposure. For results with blood and bone Pb level, there is uncertainty regarding the specific level, timing, frequency, and duration of Pb exposure associated with psychopathological effects.

The epidemiologic evidence for Pb-associated psychopathological effects is supported by the coherence with findings in rodents that dietary lactational Pb exposure with or without additional post-lactational exposure resulted in depression-like behavior and emotionality in rodents, with some evidence at blood Pb levels relevant to this ISA (13, 17 $\mu\text{g/dL}$) ([Beaudin et al., 2007](#); [Dyatlov and Lawrence, 2002](#)). Other studies found Pb-induced increases in depression-like behavior in animals with higher than relevant blood Pb levels ([Section 4.3.4.2](#)). Further support for Pb-associated increases in psychopathological effects in adults is provided by evidence that describes modes of action, including Pb-induced changes in the HPA axis ([Section 4.3.2.3](#)) and dopaminergic and GABAergic systems ([Sections 4.2.2.2, 4.3.10.4, and 4.3.10.8](#)), which are found to affect mood and emotional state.

In conclusion, cross-sectional studies in a few populations demonstrate associations of higher concurrent blood, patella, or tibia Pb levels with self-reported symptoms of depression and anxiety in adults. The examination of multiple exposures and outcomes in the available studies does not provide strong indication of biased reporting of psychopathological effects by adults specifically with higher Pb exposures. In adults, Pb-associated increases in symptoms of depression and anxiety were found with adjustment for age, SES, education, and in the NAS, daily alcohol intake and pack-years smoking. The biological plausibility for epidemiologic evidence is provided by observations of depression-like behavior or emotionality in animals with dietary lactational Pb exposure, with some evidence at blood Pb levels relevant to this ISA and by toxicological evidence supporting modes of action, including Pb-induced changes in the HPA axis and dopaminergic and GABAergic systems. The associations of blood and bone Pb level with symptoms of psychopathological effects found in the few studies of adults without occupational Pb exposure as well as the biological plausibility provided by the coherence with findings from a few rodent studies and evidence for underlying modes of action, but uncertainties related to residual confounding of bone Pb results by age in epidemiologic studies are sufficient to conclude that a causal relationship is likely to exist between Pb exposure and psychopathological effects in adults.

4.3.15.10 Evidence for Auditory Function Decrements in Adults

The evidence is suggestive of a causal relationship between Pb exposure and auditory function decrements in adults based primarily on findings from a few epidemiologic studies. Key evidence in humans is provided by the recent analysis of NAS males in which a 15 µg/g higher tibia Pb level at mean age 64.9 years was associated with a 0.05 dB/year (95% CI: 0.017, 0.083) increase in hearing threshold for a pure tone average frequency ([Park et al., 2010](#)). Results were adjusted for baseline age, race, education, occupational noise, BMI, pack-years smoking, noise notch, diabetes, and hypertension. Although the generalizability of results in this primarily white population of men is limited, high follow-up participation and the examination of multiple exposures and outcomes in this cohort reduces the likelihood that findings are biased by selective participation of men specifically with higher Pb exposures. Bone Pb levels were measured up to 20 years after the initial hearing measurement; however, tibia Pb level is considered an indicator of cumulative Pb exposure since the half-life of Pb in bone is on the order of decades ([Section 3.3](#)). Bone Pb levels increase with age, and although age was included as a model covariate, residual confounding by age is possible. Supporting evidence was provided by a recent case-control study of adults attending a hospital for occupational health exams. Despite limitations of a nonrandom population and uncertain

comparability of controls, the examination of multiple metals reduces the likelihood of biased participation by higher Pb exposure. Higher concurrent blood Pb level was associated with hearing loss with adjustment for several factors including blood levels of Mn, As, and Se ([Chuang et al., 2007](#)).

Pb-associated increases in hearing thresholds or latencies in brainstem auditory evoked potentials were found in adult monkeys ages 8-13 years with lifetime Pb exposure in drinking water from gestation or birth ([Rice, 1997](#); [Lilienthal and Winneke, 1996](#)). A recent study found small increases in hearing thresholds in monkeys at age 13 years with Pb exposure only during gestation or lactation (to age 5.5 months) ([Laughlin et al., 2009](#)). However, these effects were found with higher blood Pb levels (i.e., at peak or testing 33-150 µg/dL) than those relevant to this ISA; thus, the biological plausibility for epidemiologic observations is unclear.

In conclusion, the evidence provided by the analysis of NAS men for associations of higher tibia Pb level with a higher rate of elevations in hearing threshold over 20 years but uncertainties related to effects on auditory function in adult animals with relevant Pb exposures, is suggestive of a causal relationship between Pb exposure and auditory function decrements in adults.

4.3.15.11 Evidence for Visual Function Decrements in Adults

The effects of relevant Pb exposures on visual function related to CNS changes, specifically in adults have been examined in some toxicological studies, but epidemiologic evidence is sparse. Studies in adult animals show differential effects on ERGs, depending on the timing and concentration of Pb exposure. Although studies have linked Pb-induced ERG changes to changes in rod cell proliferation, retinal cell apoptosis, and dopamine, the implications of findings are unclear because the functional relevance of the observed ERG changes is unclear. Decreased visual acuity was found in adult monkeys with lifetime (to age 7-9 years) Pb exposure but at higher concentrations than those relevant to this ISA, 50-115 µg/dL ([Rice, 1998](#)). A case-control study found higher Pb concentrations in retinal tissue of macular degeneration adult cases ([Table 4-17](#)) but was limited by the inability to establish directionality of effects, the uncertain representativeness of the control population, and the lack of rigorous statistical analysis or examination of potential confounding factors. Because the available epidemiologic and toxicological evidence is of insufficient quantity, quality, and consistency, the evidence is inadequate to determine that a causal relationship exists between Pb exposure and visual function decrements in adults.

4.3.15.12 Evidence for Neurodegenerative Diseases in Adults

Epidemiologic and toxicological studies combined produced inconclusive evidence for Alzheimer's disease and ALS but found associations between indicators of Pb exposure and neurodegenerative diseases such as Parkinson's disease and essential tremor. However, study limitations as described below limit the implications of findings.

The few case-control studies of essential tremor found higher concurrent blood Pb levels in cases than controls ([Section 4.3.9.4](#)). A common limitation of these studies was the potential for reverse causation. Reduced physical activity among cases could result in greater bone turnover and greater release of Pb from bones into blood in cases than controls. Some case-control studies found adults with Parkinson's disease to have higher bone Pb levels, which are not likely to increase with decreases in physical activity ([Weisskopf et al., 2010](#); [Coon et al., 2006](#)). While some of these studies of Parkinson's disease and essential tremor considered potential confounding by factors such as age, sex, race, education, and alcohol consumption, they did not consider Mn co-exposure. Epidemiologic findings for Parkinson's disease are supported by limited available mode of action evidence for Pb-induced decreased dopaminergic cell activity in the substantia nigra, which contributes to the primary symptoms of Parkinson's disease.

The few case-control studies of Alzheimer's disease did not find higher prevalence of occupational Pb exposure or higher brain Pb levels in cases but did not measure Pb in blood or bone ([Section 4.3.9.1](#)). Toxicological studies indicated that gestational-lactational or infancy Pb exposure of monkeys and rats (but not adult-only exposure in rats) induced pathologies that underlie Alzheimer's disease, including the formation of amyloid plaques and neurofibrillary tangles in the brains of aged animals ([Section 4.3.9.1](#)). While these results suggest the need to consider early-life Pb exposure in epidemiologic studies, some indicate that effects may be attributable to the high Pb exposure concentrations tested, i.e., producing blood Pb levels >40 µg/dL in rats ([Li et al., 2010b](#); [Basha et al., 2005](#)). Studies of ALS have not consistently found higher Pb biomarker levels or history of occupational Pb exposure among ALS cases and controls ([Section 4.3.9.2](#)), and a recent study found that higher tibia and patella Pb levels were associated with longer survival time among ALS cases ([Kamel et al., 2008](#)).

In conclusion, while evidence is inconclusive for ALS and Alzheimer's disease, a few case-control studies each found higher blood Pb levels in adults with essential tremor and higher bone Pb levels in adults with Parkinson's disease. Because of the inconclusive evidence for some diseases and limitations such as reverse causation for essential tremor and the lack of consideration for potential confounding by Mn exposure for both essential tremor and Parkinson's disease, the evidence is inadequate to determine that there is a causal relationship between Pb exposure and neurodegenerative diseases.

Table 4-17 Summary of Evidence Supporting Nervous System Causal Determinations.

Attribute in Causal Framework ^a	Key Evidence ^b	References ^b	Pb Biomarker Levels Associated with Effects ^c
Cognitive Function Decrements in Children - Causal			
Consistent associations from multiple, high quality epidemiologic studies with relevant blood Pb levels	Evidence from prospective studies for decrements in FSIQ in association with prenatal, earlier childhood, peak, concurrent, lifetime average blood Pb levels and tooth Pb levels in children ages 4-17 yr in multiple U.S. locations, Mexico, Europe, Australia	Canfield et al. (2003a), Bellinger et al. (1992), Jusko et al. (2008), Dietrich et al. (1993b), Schnaas et al. (2006), Wasserman et al. (1997), Tong et al. (1996), Lanphear et al. (2005) Plus Table 4-3 , Section 4.3.2.1	Blood Pb (various time periods & lifestages): Means 3-16 µg/dL With consideration of peak or early childhood blood Pb levels: Means 3-8 µg/dL for concurrent (age 4, 5 yr), age 2 yr
	Evidence from prospective studies for lower scores on tests of executive function and academic performance in association with earlier childhood or lifetime average blood Pb levels or tooth Pb levels in children ages 5-20 yr in multiple U.S. locations, U.K, New Zealand. Associations less consistent for learning and memory.	Canfield et al. (2004), Stiles and Bellinger (1993), Miranda et al. (2009 ; 2007a), Fergusson et al. (1997 , 1993), Leviton et al. (1993), Chandramouli et al. (2009) Sections 4.3.2.3 , 4.3.2.4 , 4.3.2.5	Blood Pb (various time periods & lifestages): Means 4.8-7.2 µg/dL, Groups with early childhood blood Pb 2-16 µg/dL and 5-10 µg/dL Tooth Pb (ages 6-8 yr): means 3.3, 6.2 µg/g
	Supporting evidence from cross-sectional studies of children ages 3-16 yr, but most did not consider potential confounding by parental caregiving quality. Includes large NHANES III analysis.	Surkan et al. (2007), Kim et al. (2009b), Roy et al. (2011), Lanphear et al. (2000), Froehlich et al. (2007), Chiodo et al. (2007 ; 2004)	Concurrent (ages 3-16 yr) blood Pb : Means 1.7-12 µg/dL, Group (ages 6-10 yr) with blood Pb 5-10 µg/dL
	Outcomes assessed using widely-used, structured questionnaires.		
	Several studies indicate supralinear C-R relationship, with larger decrements in cognitive function per unit increase in blood Pb at lower blood Pb levels in children ages 5-10 yr	Canfield et al. (2003a), Bellinger et al. (1992), Jusko et al. (2008), Kordas et al. (2006), Lanphear et al. (2005) Plus Table 4-16	Groups with peak blood Pb <10 µg/dL: concurrent mean 3.3 µg/dL, age 2 year mean 3.8 µg/dL

Table 4-17 (Continued): Summary of Evidence Supporting Nervous System Causal Determinations.

Attribute in Causal Framework ^a	Key Evidence ^b	References ^b	Pb Biomarker Levels Associated with Effects ^c
Additional epidemiologic evidence to help rule out chance, bias, and confounding with reasonable confidence	Several epidemiologic studies found associations with adjustment for SES, maternal IQ and education, HOME score. Several adjust for birth weight, smoking. A few, nutritional factors. Epidemiologic studies had population-based recruitment, most with moderate to high follow-up participation not conditional on blood or tooth Pb level or cognitive function. Pooled and meta-analyses demonstrate the consistency of association	Table 4-3, Table 4-5; Table 4-8, Table 4-9, Sections 4.3.2.1, 4.3.2.3, 4.3.2.4, and 4.3.2.5 Lanphear et al. (2005), Pocock et al. (1994), Schwartz (1994)	
Consistent evidence in animals with relevant exposures to help rule out chance, bias, and confounding with reasonable confidence	Impaired learning and associative ability in juvenile and adult animals as indicated by performance in tasks of visual discrimination, water maze, y maze, and operant conditioning with schedules of reinforcement with relevant dietary Pb exposure.	Stangle et al. (2007), Niu et al. (2009), Cory-Slechta et al. (2010), Altmann et al. (1993), Section 4.3.2.3	Blood Pb (after prenatal/ lactation, lactation only, prenatal/lifetime Pb exposure): 10-25 µg/dL
	Impaired learning, memory, executive function in adult monkeys as indicated by poorer performance on delayed spatial alternation and spatial discrimination reversal learning tasks with dietary Pb exposures.	Gilbert and Rice (1987), Rice and Karpinski (1988), Sections 4.3.2.3 and 4.3.2.4	Blood Pb (after lifetime Pb exposure from birth): 15, 25 µg/dL

Table 4-17 (Continued): Summary of Evidence Supporting Nervous System Causal Determinations.

Attribute in Causal Framework ^a	Key Evidence ^b	References ^b	Pb Biomarker Levels Associated with Effects ^c
Evidence describes mode of action:	Decreased neurogenesis in hippocampus DG (involved in LTP and learning). Decreased NMDAR (involved in integration of new neurons into existing neuronal pathways). Decreased neurite outgrowth.	Sections 4.3.10.9 and 4.3.10.10	
Impaired neuron development	Found in animals with dietary gestational-lactational, lactational, post-lactational (3-8 weeks), lifetime from gestation Pb exposures.		
Synaptic changes	Decreased synaptic development. Changes in synaptic protein composition. Decreased ATP and AchE, which both mediate neurotransmission. Found in animals with dietary gestational with or without additional lactational Pb exposures.	Section 4.3.10.4	
LTP	Decreased magnitude, increased threshold of LTP with gestational-lactational or lifetime Pb exposure.	Sections 4.3.12 , 4.3.10.7 , 4.3.10.8	
Neurotransmitter changes	Changes in dopamine metabolism. Increased sensitivity of dopamine receptor. Increased catecholamine transmission in cerebral cortex, cerebellum, hippocampus. Decreased glutamate and expression of glutamate receptor, NMDAR. Found in animals with dietary gestational-lactational, lactational, or post-lactational Pb exposure.	Section 4.3.10.8	

Table 4-17 (Continued): Summary of Evidence Supporting Nervous System Causal Determinations.

Attribute in Causal Framework ^a	Key Evidence ^b	References ^b	Pb Biomarker Levels Associated with Effects ^c
Externalizing Behaviors: Attention, Impulsivity, and Hyperactivity in Children – Causal			
Consistent associations from multiple, high quality epidemiologic studies with relevant blood Pb levels	Evidence from prospective studies for attention decrements and hyperactivity in association with prenatal (maternal or cord), early childhood, and lifetime avg blood Pb and tooth Pb levels in children ages 7-17 yr and young adults 19-20 yr in U.S., U.K., Australia, New Zealand.	Burns et al. (1999), Ris et al. (2004), Fergusson et al. (1993), Bellinger et al. (1994a), Chandramouli et al. (2009), Leviton et al. (1993) Section 4.3.3.1	Blood Pb: Means 6.8, 8.3 µg/dL (prenatal maternal or cord), 8.3 µg/dL age 6 yr, 13.4 age 3-60 month, 14 µg/dL (lifetime avg to age 11-13 yr) Group with age 30 mo >10 µg/dL Tooth Pb (ages 6-8 yr): Means: 3.3, 6.2 µg/g
	Association not found with ratings of attention problems in children ages 4-5 yr in whom ratings may be measured less reliably	Wasserman et al. (2001) Section 4.3.3.1	Blood Pb: Mean 7.2 µg/dL for lifetime (to age 4-5 yr) avg
	Associations found with neuropsychological tests (CPT) and teacher and parent ratings using widely-used, structured questionnaires.		
Consistent associations from multiple, high quality epidemiologic studies with relevant blood Pb levels (Continued)	Supporting evidence from higher quality cross-sectional studies (consideration for several potential confounding factors) for associations of concurrent blood Pb level with attention decrements, impulsivity, and hyperactivity in children ages 5-7.5 yr. Some populations had high prenatal drug or alcohol exposure.	Chiodo et al. (2007 ; 2004) Plusquellec et al. (2010) Section 4.3.3.1	Concurrent (ages 5-7.5 yr) blood Pb: Means 5.0-5.4 µg/dL
	No association in Rochester cohort, age 4.5 yr.	Canfield et al. (2003b)	Concurrent (age 4.5 yr) blood Pb: Mean 6.5 µg/dL
	Associations found in most other cross-sectional studies with less extensive consideration for potential confounding	Section 4.3.3.1 , Table 4-11	Concurrent (ages 3-17 yr) blood Pb: Means 1-12 µg/dL
Additional epidemiologic evidence to help rule out chance, bias, and confounding with reasonable confidence	Most prospective and some cross-sectional studies found associations with adjustment for SES, maternal education, and parental caregiving quality. Some also considered parental IQ, smoking, birth outcomes. A few considered substance abuse, nutritional factors. Studies had population-based recruitment with moderate to high follow-up participation not conditional on blood or tooth Pb level.	Section 4.3.3.1 , Table 4-11	

Table 4-17 (Continued): Summary of Evidence Supporting Nervous System Causal Determinations.

Attribute in Causal Framework ^a	Key Evidence ^b	References ^b	Pb Biomarker Levels Associated with Effects ^c
Consistent evidence in animals with relevant exposures to help rule out chance, bias, and confounding with reasonable confidence	Impulsivity indicated by premature responses, increased response perseveration, decreased pause time between events on tests with FI, FR/waiting for reward, or Differential reinforcement of low response rate in rodents and monkeys with relevant dietary postnatal Pb exposures.	Rossi-George et al. (2011), Brockel and Cory-Slechta (1999b , 1998), Rice and Gilbert (1985), Gilbert and Rice (1987) Section 4.3.3.1	Blood Pb: 15, 25 µg/dL in monkeys with lifetime exposure, 11-16 µg/dL in rats with lifetime exposure from gestation; 11-29 µg/dL in rats with 3, 7 mo post-weaning exposure
	Inconsistent evidence for sustained attention in rats and monkeys with relevant postnatal dietary Pb exposures. Increased activity in male mice found only with Pb and amphetamine co-exposure.	Sustained attention: Gilbert and Rice (1987), Brockel and Cory-Slechta (1999a) Hyperactivity: Leasure et al. (2008) Section 4.3.3.1	Blood Pb: 15, 25 µg/dL in monkeys after lifetime (after birth) exposure; 16, 28 µg/dL in rats after 34-day post-weaning exposure
Evidence describes mode of action	Same as above for cognitive function in children		

Table 4-17 (Continued): Summary of Evidence Supporting Nervous System Causal Determinations.

Attribute in Causal Framework ^a	Key Evidence ^b	References ^b	Pb Biomarker Levels Associated with Effects ^c
Externalizing Behaviors: Conduct Disorders in Children and Young Adults (e.g., delinquent, aggressive, antisocial behavior, and criminal offenses) – Likely Causal			
Consistent results from a few high-quality epidemiologic studies with relevant blood or tooth Pb levels	Evidence from prospective studies for higher parent and teacher ratings of aggressive, antisocial, delinquent behavior in children ages 8-17 yr in U.S., U.K., Australia in association with earlier childhood or lifetime average blood Pb levels.	Dietrich et al. (2001), Burns et al. (1999), Chandramouli et al. (2009), Section 4.3.3.2	Blood Pb: lifetime (to age 11-13 yr) avg mean 14 µg/dL, age 6 year mean 12 µg/dL, group with age 30 month blood Pb >10 µg/dL
	Evidence from prospective studies for criminal offenses in young adults ages 19-24 yr in Cincinnati and New Zealand in association with earlier childhood blood or tooth Pb levels.	Wright et al. (2008), Fergusson et al. (2008), Section 4.3.3.2	Age 6 yr blood Pb mean 6.8 µg/dL Tooth Pb (age 6-8 yr) mean: 6.2 µg/g
	Association found in Yugoslavia cohort at ages 4-5 years, when behavior ratings may be less reliable.	Wasserman et al. (2001)	Mean lifetime (to ages 4-5 yr) avg: 7.2
	Studies had moderate to high follow-up participation, not conditional on blood Pb level.	Table 4-12	
	Most studies considered potential confounding by SES, parental IQ and education, other SES factors, parental caregiving quality, family functioning, parental smoking or substance abuse.		
	Supporting evidence from cross-sectional studies of children ages 7-15 years, including NHANES analysis and study of children in Detroit, MI that had extensive consideration for potential confounding.	Braun et al. (2008) Chiodo et al. (2007) Section 4.3.3.2	Groups ages 8-17 years with concurrent blood Pb 0.8-10 µg/dL Concurrent (age 7 year) blood Pb mean: 5.0 µg/dL
	Consistency supported by meta-analysis indicating similar effect estimates by study design, potential confounding factors considered	Marcus et al. (2010) Section 4.3.3.2	Blood Pb range of study means (concurrent, lifetime avg): 1.0-26 µg/dL
	Teacher and parental ratings derived from widely-used, structured questionnaires.		

Table 4-17 (Continued): Summary of Evidence Supporting Nervous System Causal Determinations.

Attribute in Causal Framework ^a	Key Evidence ^b	References ^b	Pb Biomarker Levels Associated with Effects ^c
But, uncertainty because of inconsistent animal evidence with relevant exposures and small number of epidemiologic studies	Aggression observed in adult hamsters with gestational-lifetime dietary Pb exposure. Other evidence in adult animals with similar duration exposure inconsistent. Aggression generally not found in juvenile animals with lactational Pb exposure. No specific mode of action examined with Pb exposure.	Delville (1999) Section 4.3.3.2	Blood Pb after gestational-lifetime exposure: 10-15 µg/dL
Internalizing Behaviors in Children (e.g., withdrawn behavior, symptoms of depression, anxiety, fearfulness) – Likely Causal			
Consistent results from a few high-quality epidemiologic studies with relevant exposures	Evidence from prospective studies for higher ratings of internalizing behaviors in children ages 8-13 yr in Boston and Port Pirie cohorts in association with tooth or lifetime average blood Pb levels. Associations also found in children age 4-5 yr in Yugoslavia in association with lifetime average blood Pb level; stronger association found for delinquent behavior. Studies had population-based recruitment with moderate follow-up participation. Participation not conditional on tooth/blood Pb levels and behavior. Associations found with teacher and parent ratings on widely used, structured questionnaires. Inconsistent associations in cross-sectional studies that had extensive consideration for potential confounding and examined relatively low concurrent blood Pb levels.	Burns et al. (1999), Bellinger et al. (1994b) Section 4.3.4.1 Wasserman et al. (2001) Chiodo et al. (2004) Plusquellec et al. (2010) Section 4.3.4.1	Blood Pb lifetime (to age 11-13 yr) average mean: ~14 µg/dL Tooth Pb (age 6 yr) mean: 3.4 µg/g Blood Pb lifetime (to age 4-5 yr) average mean: 7.2 µg/dL Concurrent blood Pb means (ages 5, 7 yr): 5, 5.4 µg/dL
And, supporting animal evidence with relevant exposures from a few studies	Dietary lactational Pb exposure increased emotionality, depression-like behavior in juvenile rodents	Beaudin et al. (2007) Dyatlov and Lawrence (2002) Section 4.3.4.2	Blood Pb at PND52 after PND1-PND30 exposure: 13 µg/dL Blood Pb at PND22 after PND1-PND22 exposure: 17 µg/dL
Some evidence describing mode of action	Pb-induced changes in HPA axis and dopaminergic and GABAergic systems	Sections 4.3.2.3, 4.2.2.2, 4.3.10.4, 4.3.10.8	

Table 4-17 (Continued): Summary of Evidence Supporting Nervous System Causal Determinations.

Attribute in Causal Framework ^a	Key Evidence ^b	References ^b	Pb Biomarker Levels Associated with Effects ^c
But, uncertainty regarding potential confounding	Epidemiologic associations found with adjustment for SES, birth outcomes, parental education. Few studies adjusted for or considered HOME score. Uncertainty regarding potential confounding by parental caregiving quality.	Burns et al. (1999), Bellinger et al. (1994b), Wasserman et al. (2001) Chiodo et al. (2004) Plusquellec et al. (2010) Section 4.3.4.1	
Auditory Function Decrements in Children – Likely Causal			
Consistent findings from a few high-quality epidemiologic studies with relevant blood Pb levels	Prospective study found associations of prenatal (maternal), neonatal, yearly age 1 to 5 yr, lifetime avg blood Pb levels with poorer auditory processing in children at age 5 yr in Cincinnati. Information was not provided on participation rates. Results were adjusted for SES, HOME score, birth outcomes, obstetrical complications, maternal smoking. Several other factors considered. Supporting evidence from cross-sectional studies for increased hearing thresholds in children ages 4-19 yr participating in NHANES and HHANES in association with higher concurrent blood Pb levels. Examination of multiple exposures and outcomes in NHANES and HHANES reduces likelihood of selection bias specifically by blood Pb levels. Studies considered potential confounding by age, sex, race, income, parental education, nutritional factors.	Dietrich et al. (1992) Section 4.3.6.1 Schwartz and Otto (1991, 1987) Section 4.3.6.1	Blood Pb means: neonatal (10 day) 4.8 µg/dL, yearly age 1 to 5 year 10.6-17.2 µg/dL, lifetime (to age 5 yr) avg NR Blood Pb median: HHANES: 8 µg/dL NHANES: NR
But, uncertainty because of lack of animal evidence in juveniles and at relevant exposures	Increased hearing thresholds, decreased auditory evoked potentials in adult monkeys (ages 8-13 years) with lifetime dietary Pb exposure. Changes in neurotransmitters in regions of brain involved in auditory integration in mice with gestational-lactational Pb exposure	Rice (1997) Lilienthal and Winneke (1996) Section 4.3.6.1 Fortune et al. (2009) Section 4.3.10.8	Blood Pb after lifetime exposure: 33-150 µg/dL Blood Pb after gestational-lactational exposure: 8.2, 42 µg/dL

Table 4-17 (Continued): Summary of Evidence Supporting Nervous System Causal Determinations.

Attribute in Causal Framework ^a	Key Evidence ^b	References ^b	Pb Biomarker Levels Associated with Effects ^c
Visual Function Decrements in Children - Inadequate			
The available evidence is of insufficient quantity, quality, or consistency	High maternal 1 st trimester blood Pb associated with supernormal ERG in a study of children. No potential confounding considered. Uncertain functional relevance of ERG findings.	Rothenberg et al. (2002b) Section 4.3.6.2	Group with prenatal maternal blood Pb 10.5-32.5 µg/dL
	Higher than relevant Pb exposures did not affect visual acuity in infant monkeys but decreased visual acuity in adult monkeys.	Laughlin et al. (2008) Section 4.3.6.2	Blood Pb after postnatal day 8-week 26 exposure: 35-40 µg/dL
	Examination of retinal ERGs limited to adult rats (see below for Visual Function Decrements in Adults)	Section 4.3.6.2	
Motor Function Decrements in Children – Likely Causal			
Consistent findings from a few high-quality epidemiologic studies with relevant blood Pb levels	Evidence from prospective studies for fine and gross motor function decrements in children ages 4.5-17 yr in Cincinnati, Yugoslavia in association with neonatal, earlier childhood, concurrent, lifetime avg blood Pb levels. High follow-up participation, no selective attrition in Cincinnati cohort, higher loss-to-follow-up in Yugoslavia cohort with lower maternal IQ, HOME. Both studies adjusted for maternal IQ, parental education, SES, HOME score Studies used various, widely-used tests to assess outcomes.	Ris et al. (2004), Dietrich et al. (1993a), Bhattacharya et al. (2006; 1995), Wasserman et al. (2000b) Section 4.3.7	Blood Pb means Cincinnati: neonatal (10 day) 4.8 µg/dL, concurrent (age 6 yr) 11.6 µg/dL, lifetime (to age 15-17 yr) avg 12.3 µg/dL, age 0-5 yr avg 11.7 µg/dL Yugoslavia: NR
	Mixed evidence for lower (concurrent) blood Pb levels from cross-sectional studies that considered several potential confounding factors.	Section 4.3.7	
	No decrease in motor function found in children ages 6-10 yr in New England.	Surkan et al. (2007)	Concurrent blood Pb mean: 2.2 µg/dL
	Decrease found in Inuit children ages 4-6 years in Canada.	Despres et al. (2005),	Concurrent blood Pb mean: 4.1 µg/dL

Table 4-17 (Continued): Summary of Evidence Supporting Nervous System Causal Determinations.

Attribute in Causal Framework ^a	Key Evidence ^b	References ^b	Pb Biomarker Levels Associated with Effects ^c
But, uncertainty because of limited animal evidence at relevant exposures	Poorer balance (fell off rotarod more quickly) in adult mice with gestational-lactation dietary Pb exposure Inconsistent results with higher than relevant postnatal exposures	Leasure et al. (2008) Section 4.3.7 Section 4.3.7	Peak blood Pb after gestational -lactational exposure ~10 µg/dL Blood Pb after 2-7 week postnatal Pb exposure >59 µg/dL
Cognitive Function Decrements in Adults – Likely Causal			
Consistent findings from high-quality epidemiologic studies with relevant bone Pb levels	Prospective analyses in NAS cohort of white men and BMS cohort of men and women of diverse ethnicities found cognitive function decrements over 2 to 4 years in association with bone Pb levels. Baseline participation rates differed but high follow-up participation, not conditional on bone Pb levels. Different potential confounding factors considered. Adjustment for age and education in both cohorts, SES in BMS, smoking and alcohol use in NAS. Supporting cross-sectional evidence from NAS, BMS, and also women in Nurses' Health Study with adjustment for additional potential confounding factors, including dietary factors, medications, physical activity, comorbid conditions. Associations with concurrent blood Pb level found in men and women participating in NHANES in certain age and genetic variant groups but not consistently.	Weisskopf et al. (2007b), Bandeem-Roche et al. (2009) Table 4-10 and Section 4.3.2.7 Nurses Healthy Study: Weuve et al. (2009) Section 4.3.2.7 Table 4-10 Krieg et al. (2009), Krieg et al. (2010), Krieg and Butler (2009) Section 4.3.2.7	Baseline means: 19, 20 µg/g tibia 25 µg/g patella Concurrent means: 11 µg/g tibia 13 µg/g patella Concurrent blood Pb means: 3-4 µg/dL
	Some studies found associations in former and current Pb-exposed workers with relevant blood Pb levels and bone Pb levels. Most studies adjusted for age and education. Some also adjusted for depression and/or alcohol use; none considered other occupational exposures such as Mn. Outcomes assessed using various but widely used, structured instruments.	Dorsey et al. (2006), Schwartz et al. (2002) Stewart et al. (2002) Section 4.3.2.7	Concurrent blood Pb means: 31 µg/dL Peak tibia Pb mean: 26 µg/g.

Table 4-17 (Continued): Summary of Evidence Supporting Nervous System Causal Determinations.

Attribute in Causal Framework ^a	Key Evidence ^b	References ^b	Pb Biomarker Levels Associated with Effects ^c
Consistent evidence in animals with relevant exposures	Impaired learning, memory, and executive function in monkeys ages 7-10 years with lifetime dietary Pb exposures from birth. Impaired learning in animals with lifetime dietary Pb exposures starting in gestation.	Rice and Karpinski (1988), Gilbert and Rice (1987) Section 4.3.2.3 See above for cognitive function in children.	Blood Pb at age 200 days: 15, 25 µg/dL
Evidence clearly describes mode of action	Same as above for cognitive function in children		
But, important uncertainty remains	Few prospective studies available to address reverse causation Uncertainty regarding potential residual confounding of bone Pb results by age		
Psychopathological Effects in Adults (e.g., symptoms of depression, anxiety, panic disorder) – Likely Causal			
Consistent findings from a few high-quality epidemiologic studies with relevant blood and bone Pb levels	A few population-based cross-sectional studies indicate associations of higher concurrent blood, patella, or tibia Pb level with increased reporting of symptoms of depression, anxiety, panic disorder in adults. Studies had high participation rates and examined multiple exposures and outcomes and include NHANES analysis of 1,987 adults. Studies used widely used, structured instruments to assess outcomes but not diagnosed conditions.	Bouchard et al. (2009), Rhodes et al. (2003), Rajan et al. (2008) Section 4.3.5.1 Bouchard et al. (2009) Section 4.3.5.1	Concurrent blood Pb mean: 6 µg/dL Tibia Pb mean: 22 µg/g Patella Pb mean: 31, 32 µg/g Group with concurrent blood Pb ≥ 2.11 µg/dL
And, some supporting animal evidence with relevant exposures	Same as above for internalizing behaviors in children.		
But, uncertainty regarding potential confounding	Associations found with adjustment for education, age, and SES. Mn not considered. Also alcohol use and pack-years smoking in NAS. However, uncertainty regarding generalizability because of limited number of studies and potential for residual confounding of bone Pb results by age.	Section 4.3.5.1 Rhodes et al. (2003), Rajan et al. (2007)	

Table 4-17 (Continued): Summary of Evidence Supporting Nervous System Causal Determinations.

Attribute in Causal Framework ^a	Key Evidence ^b	References ^b	Pb Biomarker Levels Associated with Effects ^c
Auditory Function Decrements in Adults – Suggestive			
Limited epidemiologic evidence with relevant bone or blood Pb levels, one high-quality study	Prospective study found association between tibia Pb level and higher rate of increase in hearing threshold over 23 yr in NAS.	Park et al. (2010) Section 4.3.6.1	Tibia Pb mean: 22.5 µg/g, measured near end of follow-up
	Population comprises only males, primarily white, but study examines multiple exposures and outcomes and has high follow-up participation. Results adjusted for age, race, BMI, education, diabetes, hypertension, smoking, occupational noise.		
	Supporting evidence from case-control study finding higher blood Pb levels in workers from various occupations with hearing loss with adjustment for age, smoking, alcohol consumption, years of noise exposure, blood Mn, As, Se	Chuang et al. (2007) Section 4.3.6.1	Concurrent blood Pb geometric mean in cases: 10.7 µg/dL
Lack of animal evidence at relevant exposures	Increased hearing thresholds, decreased auditory evoked potentials in adult monkeys (ages 8-13 years) with gestational, lactational, lifetime dietary Pb exposure.	Rice (1997) Lilienthal and Winneke (1996) Laughlin et al. (2009) Section 4.3.6.1	Blood Pb after gestational, lactational, or lifetime exposure: 33-150 µg/dL
Visual Function Decrements in Adults - Inadequate			
The available evidence is of insufficient quantity, quality, or consistency	Epidemiologic evidence limited to case-control study of higher Pb in retinal tissue of macular degeneration adult cases. Potential confounding not considered.	Erie et al. (2009)	Median Pb in retina tissue Cases: 12.0 ng/g Controls: 8.0 ng/g
	Higher than relevant Pb exposures decreases visual acuity in adult monkeys but not in infant monkeys.	Rice et al. (1998)	Blood Pb: 50-115 µg/dL with lifetime exposure to age 7-9 yr
	Supernormal or subnormal ERGs in adult rats depending on timing and dose of Pb exposure that were linked to changes in rod cell proliferation, retinal cell apoptosis, dopamine. Uncertain functional relevance.	Fox et al. (2008; 1997), Section 4.3.6.2	Blood Pb: 12, 24 µg/dL with gestational-lactational exposure, 19 µg/dL with lactational exposure

Table 4-17 (Continued): Summary of Evidence Supporting Nervous System Causal Determinations.

Attribute in Causal Framework ^a	Key Evidence ^b	References ^b	Pb Biomarker Levels Associated with Effects ^c
Neurodegenerative Diseases (e.g., Alzheimer's disease, ALS, Parkinson's disease, Essential tremor) – Inadequate			
The available evidence is of insufficient quantity, quality, or consistency	The few available case-control studies found higher blood or bone Pb levels or whole-body lifetime burden (PBPK modeling) in cases with Parkinson's disease and essential tremor. Results for ALS are mixed, association found with increased survival time. Studies subject to selection and recall bias and reverse causation (for blood Pb studies) that could produce artifactual associations. Some studies consider potential confounding by age, sex, smoking, alcohol use, education, and activity levels, but not Mn exposures. Case-control studies did not show associations between occupational history of Pb exposure or brain Pb levels and Alzheimer's disease.	Parkinson's disease: Section 4.3.9.3 Weisskopf et al. (2010), Coon et al. (2006) Essential tremor: Section 4.3.9.4 Louis et al. (2005; 2003), Dogu et al. (2007) ALS: Section 4.3.9.2 Kamel et al. (2008; 2005; 2002), Vinceti et al. (1997), Fang et al. (2010) Graves et al. (1991), Hariguchi et al. (2001), Section 4.3.9.1	Parkinson's disease: group with tibia Pb levels >16 µg/g, whole-body lifetime burden ≥ 81 µg/g Essential tremor: concurrent blood Pb means 2.7-3.5 µg/dL ALS: Groups with concurrent blood Pb 3-14 µg/dL, concurrent blood Pb means 2.4, 12.7 µg/dL
Some evidence describes mode of action	Amyloid plaques found in brains of adult monkeys and rodents with infancy-only Pb exposures. Effects in monkeys at ages 20-23 yr when blood Pb levels had returned to baseline. In rodents, no effect with adult-only Pb exposure. Increases in neuronal cell apoptosis found animals with Pb exposure	Section 4.3.9.1 Wu et al. (2008a) Basha et al. (2005) Section 4.3.9.5	Blood Pb with exposure to age 400 days: peak 32-36 µg/dL Blood Pb with lactational exposure: 46 µg/dL

^aDescribed in detail in [Table II](#) of the [Preamble](#).

^bDescribes the key evidence and references contributing most heavily to causal determination and, where applicable, to uncertainties or inconsistencies. References to earlier sections indicate where full body of evidence is described.

^cDescribes the Pb biomarker levels with which the evidence is substantiated and blood Pb levels in animals most relevant to this ISA.

4.4 Cardiovascular Effects

4.4.1 Introduction

The 2006 Pb AQCD ([U.S. EPA, 2006b](#)) concluded that both epidemiologic and animal toxicological studies support the relationship between increased Pb exposure and increased cardiovascular effects, in particular, increased blood pressure (BP) and increased incidence of arterial hypertension. Although fewer in number, epidemiologic studies demonstrated associations of blood and bone Pb levels with other cardiovascular diseases (CVDs) in adults, such as ischemic heart disease, cerebrovascular disease, peripheral vascular disease, and CVD-related mortality. As the cardiovascular and renal systems are intimately linked, cardiovascular effects can arise secondarily to Pb-induced renal injury ([Section 4.5](#)). Toxicological studies also provided compelling evidence supporting the biological plausibility for Pb-associated cardiovascular effects by characterizing a number of the underlying mechanisms by which Pb exposure can lead to human cardiovascular health effects. Such studies demonstrated that the Pb content in heart tissue of animals reflects the increases in blood Pb levels ([Lal et al., 1991](#)), indicating that the cardiovascular morbidity associated with blood Pb levels may represent the effects of the bioavailable Pb in the target tissue. The strongest evidence supported the role of oxidative stress in the pathogenesis of Pb-induced hypertension. Additionally, several toxicological studies characterized other pathways or cellular, molecular, and tissue events promoting the Pb-induced increase in BP. These mechanisms included inflammation, adrenergic and sympathetic activation, renin-angiotensin-aldosterone system (RAAS) activation, vasomodulator imbalance, and vascular cell dysfunction.

With regard to the concentration-response relationship, a meta-analysis of human studies found that each doubling of blood Pb level (within a range extending from 1 at the lower end to above 40 µg/dL at the upper end, measured concurrently in most studies) was associated with a 1 mmHg increase in systolic BP and a 0.6 mmHg increase in diastolic BP ([Nawrot et al., 2002](#)). On a population-wide basis, the estimated effect size could translate into a clinically significant increase in the segment of the population with the highest BP. In a moderately-sized population, a relatively small effect size thus has important health consequences for the risk of sequelae of increased BP, such as stroke, myocardial infarction, and sudden death. It was also noted that most of the reviewed studies examining bone Pb levels, biomarkers of cumulative Pb exposure, also showed increased BP ([Cheng et al., 2001](#); [Hu et al., 1996a](#)) or increased hypertension with

increasing bone Pb level ([Lee et al., 2001a](#)). Across studies, over a range of bone Pb concentrations (<1.0 to 96 µg/g), every 10 µg/g increase in bone Pb was associated with increased odds ratios of hypertension between 1.28 and 1.86. Studies observed an average increase in systolic BP of ~0.75 mmHg for every 10 µg/g increase in bone Pb concentration over a range of <1 to 52 µg/g.

With regard to etiologically-relevant timing of Pb exposure, toxicological evidence demonstrated increases in BP after long-term (>4 weeks) Pb exposure. In epidemiologic studies, cardiovascular outcomes were most often examined in cross-sectional studies with one or a limited number of Pb biomarker measurements, so uncertainty exists as to the specific Pb exposure level, timing, frequency, and duration that contributed to the observed associations. While associations of adult bone Pb (particularly tibia Pb) with health outcomes in adults are indicative of effects related to past or cumulative exposures, interpretation of similar associations involving adult blood Pb levels, especially those measured concurrently with outcomes, is complicated by the higher past exposures generally observed in U.S. adults populations. Detailed interpretation of Pb in blood and bone are provided in [Sections 3.3](#) and [3.7.3](#). Briefly, higher past Pb exposures in adults increased their bone Pb stores which contribute to current blood Pb levels through the normal process of bone remodeling, as well as periods of increased bone remodeling and loss (e.g., osteoporosis and pregnancy). Due to the long latency period for the development of increased BP and CVD, associations of cardiovascular effects with low concurrent blood Pb levels (e.g., population means 1.6-4 µg/dL) in adults may be influenced by higher past Pb exposures ([Section 3.4.1](#)).

Past air Pb concentration and blood Pb data provide context for the cardiovascular studies. [Section 2.2](#) notes that the peak U.S. use of Pb anti-knock additives in automobile gasoline and peak industrial emissions occurred between 1968 and 1972 and Pb in gasoline was finally banned from use in 1996. [Section 2.5](#) shows that the annual average of the maximum 3-month average air Pb concentration for trends monitors across the U.S. decreased from 1.3 µg/m³ in 1980 to 0.14 µg/m³ in 2010. The mean 2010 3-month rolling average for non-source monitors (i.e., monitors not in close proximity to specific sources) was an order of magnitude lower than the 2010 trends site average. Studies of blood Pb during this time period of peak air Pb concentrations in the U.S. (1968-1980) show higher blood Pb levels than 2009-2010 levels presented in [Section 3.4](#). Among subjects ages 1-74 years examined in the U.S. NHANES II study (1976-1980) by Pirkle et al. ([1994](#)), the geometric mean blood Pb level was 12.8 µg/dL. When stratified by age, studies report geometric mean blood Pb levels in adults (age 20-79 years in 1968-1980) of 13.1 – 16.2 µg/dL ([Pirkle et al., 1994](#); [Tepper and Levin, 1975](#)) and in children (age 1 mo – 6 years in 1970-1980) of 15.0-30.4 µg/dL ([Pirkle et al., 1994](#); [Billick et al., 1979](#); [Fine et al., 1972](#)).

This section reviews the published studies pertaining to the cardiovascular effects of Pb exposure in humans, experimental animals, isolated vascular tissues, and cultured vascular cells. With the large and strong existing body of evidence serving as the foundation, emphasis was placed on studies published since the 2006 Pb AQCD. Epidemiologic and toxicological studies continued to augment the evidence for increases in BP and hypertension development associated with long-term Pb exposure and expanded the evidence for the biological pathways of these effects. Epidemiologic studies strengthened the evidence for associations between Pb biomarkers and cardiovascular effects after adjusting for potential confounding factors such as age, SES, diet, alcohol use, BMI, comorbidities, and smoking. Emphasis was placed on studies that had extensive consideration for confounding and prospective study designs. The epidemiologic evidence was substantiated with results from several available prospective studies demonstrating the directionality of effects by indicating associations between Pb biomarkers and the subsequent incidence of cardiovascular health effects.

4.4.2 Blood Pressure and Hypertension

4.4.2.1 Epidemiology

The most commonly used indicator of cardiovascular morbidity was increased BP and its derived index, hypertension. Hypertension in these studies was defined as diastolic and/or systolic BP above certain cut-points or use of anti-hypertensive medicines. The BP cut-points were established by reference to informed medical opinion, but BP cut-points defining hypertension have been lowered over time, as medical knowledge has improved. Consequently, different studies using “hypertension” as a cardiovascular outcome may have assigned different cut-points, depending on the year and location of the study and the individual investigator. All of the recent studies in the current review used similar criteria for hypertension (e.g., systolic BP at or above 140, diastolic BP at or above 90, or use of anti-hypertensive medications). Studies in the medical literature show that elevated BP is associated with increased risk of CVD including coronary disease, stroke, peripheral artery disease, and cardiac failure. Coronary disease (i.e., myocardial infarction, angina pectoris, and sudden death) is the most lethal sequelae of hypertension ([Ingelsson et al., 2008](#); [Chobanian et al., 2003](#); [Pastor-Barriuso et al., 2003](#); [Prospective Studies Collaboration, 2002](#); [Kannel, 2000a, b](#); [Neaton et al., 1995](#)).

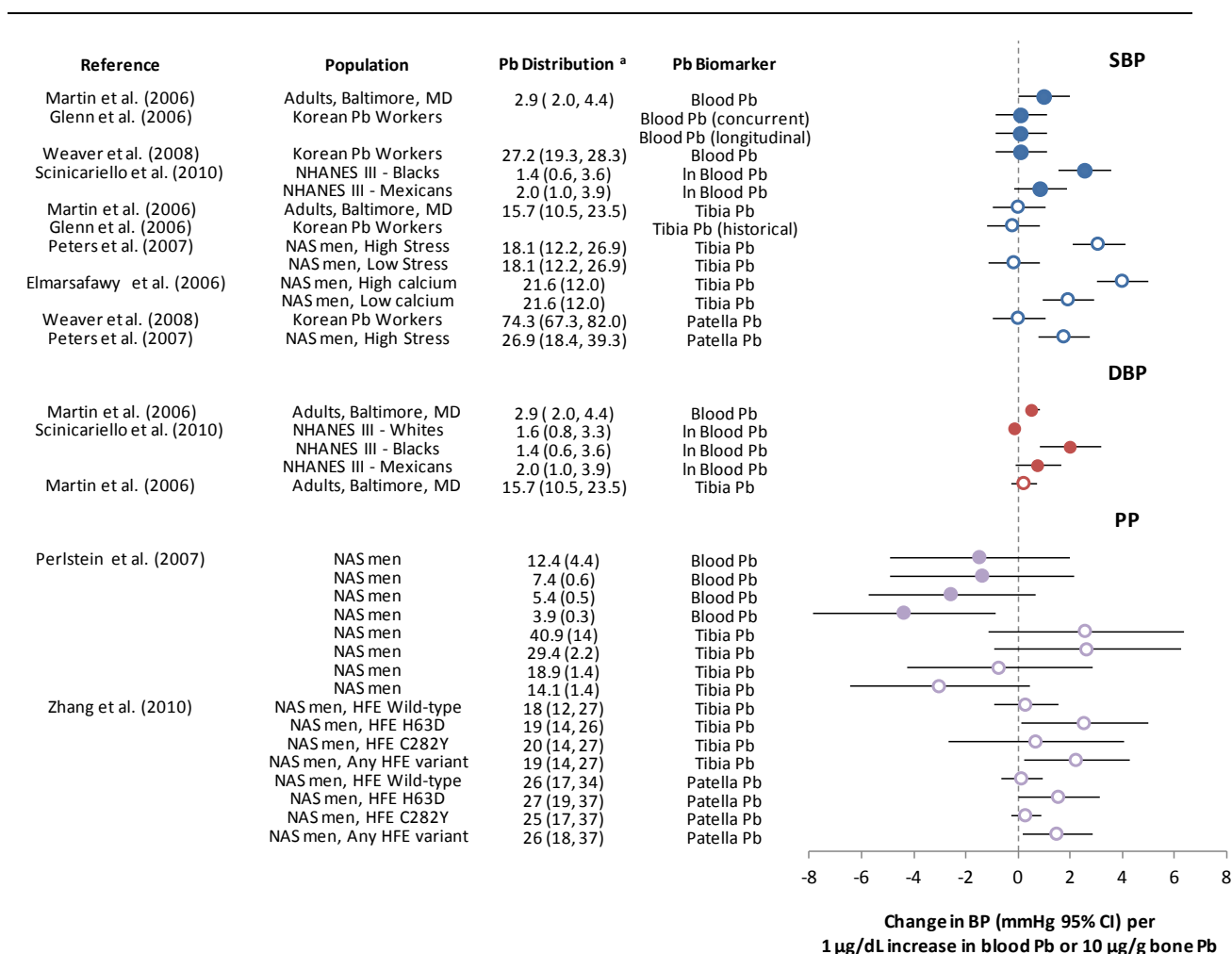
Earlier, U.S. EPA ([1990a](#)) reviewed the then available studies examining Pb exposure and BP and hypertension outcomes which included evaluation of several studies conducting analysis of the data in NHANES II (1976-80). They noted that across a range

of 7 to 34 µg/dL, no evident threshold was found below which concurrent blood Pb was not significantly related to BP. U.S. EPA ([1990a](#)) concluded that a small but positive association exists between blood Pb levels and increases in BP. Quantitatively, the relationship appeared to hold across a wide range of blood Pb values and, furthermore, an estimated mean increase of 1.5-3.0 mmHg in systolic BP appeared to occur for every doubling of blood Pb concentration in adult males and less than 1.0-2.0 mmHg for adult females. U.S. EPA ([1990a](#)) further concluded that the plausibility of these relationships observed in epidemiologic studies of human populations being of a causal nature is supported by controlled experimental animal studies demonstrating increased BP clearly attributable to Pb. Subsequently, the 2006 Pb AQCD ([U.S. EPA, 2006b](#)) reviewed the literature examining Pb exposure and effects of BP and hypertension, published after the 1990 document as discussed in [Section 4.4.1](#).

Several recent general population and occupational cohort and cross-sectional studies strengthened the evidence for associations of blood and bone Pb levels with measures of BP ([Figure 4-17](#) and [Table 4-18](#)) and with the prevalence and incidence of hypertension ([Figure 4-18](#) and [Table 4-19](#)). Further, recent studies expanded evidence, finding differences in association among racial/ethnic groups, perceived stress, diet, and genetic variants, and thus, identified populations potentially at increased risk of Pb-associated cardiovascular effects.

In a cross-sectional analysis, Martin et al. ([2006](#)) examined the associations of concurrent blood and tibia Pb levels with BP and hypertension in a large, community-based study of older adults (N = 964, age ranging from 50 to 70 years) in Baltimore, MD. Although cross-sectional in design, a key strength of this study was the extensive consideration of potential confounding variables. Four models evaluated associations for BP and hypertension. The base model included age, sex, BMI, sodium intake, potassium intake, total cholesterol, time of day, testing technician, and hypertensive medication use. Other models added SES, race/ethnicity, or both as covariates. Blood Pb but not tibia Pb level was a strong predictor of BP in all models; a 1 µg/dL increase in concurrent blood Pb level was associated with an approximately 1 mmHg increase in systolic BP and an approximately 0.5 mmHg increase in diastolic BP. Tibia Pb but not blood Pb was associated with hypertension in logistic regression models. The authors applied propensity analysis to their models to better account for the effect of other risk factors for hypertension such as race/ethnicity, age, and SES that were strongly associated with tibia Pb level. The propensity score analysis and model adjustment did not substantially change the numerical findings and conclusions (e.g., tibia Pb and hypertension were positively associated independently of race/ethnicity and SES), indicating that neither SES nor race/ethnicity confounded the association between tibia Pb level and hypertension. No evidence for effect modification by race/ethnicity was found either.

Martin et al. ([2006](#)) concluded that Pb in blood has a short term effect on BP and that Pb contributes to hypertension risk as a function of cumulative, chronic exposure (as represented as bone Pb in this population). While different aspects of Pb exposure may contribute differentially to increases in BP and hypertension, it is important to note that concurrent blood Pb levels in adults also reflect cumulative Pb exposure. Thus, its association with BP may not reflect a short term effect but may instead reflect an effect of cumulative Pb exposure.



^aPb distributions present the median (IQR), which were estimated from the mean and SD assuming a normal distribution.

^bEffect estimates were standardized to 1 µg/dL blood Pb or 10 µg/g bone Pb.

Note: In general, results are categorized by specific BP parameter, then by Pb biomarker. For categories with multiple studies, the order of the studies follows the order of discussion in the text. Results display associations (95% CI) of a 1 µg/dL increase in blood Pb level (closed circles) or 10 µg/g increase in bone Pb (open circles) with systolic BP (SBP; blue), diastolic BP (DBP; red), and pulse pressure (PP; purple) in adults.

Figure 4-17 Associations of blood and bone Pb levels with systolic BP, diastolic BP, and pulse pressure in adults.

Table 4-18 Additional characteristics and quantitative data for associations of blood and bone Pb with BP measures for studies presented in Figure 4-17.

Study	Study Population / Methodology	Parameter	Pb Data	Statistical Analysis	Effect Estimate β (95% CI)
Martin et al. (2006)	Cross-sectional 964 men and women, ages 50-70 yr, 40% African American, 55% White, 5% other, in Baltimore, MD	BP	Concurrent Mean Blood Pb: Mean (SD): 3.5 (2.3) $\mu\text{g/dL}$ African American: 3.4 (2.3) White: 3.5 (2.4) Tibia Pb: Mean (SD): 18.8 (12.4) $\mu\text{g/g}$ African American: 21.5 (12.6) White: 16.7 (11.9)	Extensive analysis of potential confounding factors. Multiple linear regression base models adjusted for age, sex, BMI, antihypertensive medication use, dietary sodium intake, dietary potassium intake, time of day, testing technician, serum total cholesterol. SES, race/ ethnicity also included in models that are presented in Figure 4-17 , and tabulated here.)	Blood Pb: SBP: 1.05 (0.53, 1.58) DBP: 0.53 (0.25, 0.81) mmHg per $\mu\text{g/dL}$ blood Pb Tibia Pb: SBP: 0.07 (-0.05, 0.14) DBP: 0.05 (-0.02, 0.08) mmHg per $\mu\text{g/g}$ bone Pb
Glenn et al. (2006)	Longitudinal 575 Pb exposed workers, ages 18-65 yr, in South Korea (10/1997-6/2001)	BP	Blood Pb mean (SD): Visit 1: 20.3 (9.6), Women Visit 2: 20.8 (10.8), Women Visit 3: 19.8 (10.7), Women Visit 1: 35.0 (13.5), Men Visit 2: 36.5 (14.2), Men Visit 3: 35.4 (15.9), Men	Multivariable models using GEE were used in longitudinal analyses. Models were adjusted for visit number, baseline age, baseline age squared, baseline lifetime alcohol consumption, baseline BMI, sex, baseline BP lowering medication use, alcohol consumption.	Model 1 (short-term) Blood Pb (longitudinal): 0.09 (0.01, 0.16) Blood Pb (concurrent): 0.08 (-0.01, 0.16) Model 4 (short and longer-term) Blood Pb (longitudinal): 0.09 (0.01, 0.16) Blood Pb (concurrent): 0.10 (0.01, 0.19) mmHg per 10 $\mu\text{g/dL}$ blood Pb

Table 4-18 (Continued): Additional characteristics and quantitative data for associations of blood and bone Pb with BP measures for studies presented in Figure 4-17.

Study	Study Population / Methodology	Parameter	Pb Data	Statistical Analysis	Effect Estimate β (95% CI)
Weaver et al. (2008)	Cross-sectional 652 current and former Pb workers in South Korea (12/1999-6/2001) Same cohort as Glenn et al. (2006)	BP	Concurrent Blood Pb: Mean (SD): 30.9 (16.7) $\mu\text{g/dL}$ Concurrent Patella Pb: Mean (SD): 75.1 (101.1) $\mu\text{g/g}$	Linear regression model adjusted for age, sex, BMI, diabetes, antihypertensive and analgesic medication use, Pb job duration, work status, tobacco and alcohol use	SBP Patella Pb: 0.0059 (-0.008, 0.02) ^a Blood Pb: 0.1007 (0.02, 0.18) ^a mmHg per 1 $\mu\text{g/dL}$ blood Pb or 1 $\mu\text{g/g}$ patella Pb Interaction between blood Pb/patella Pb with ALAD and vitamin D receptor polymorphisms not significant.
Perlstein et al. (2007)	Cross-sectional 593 elderly men (mostly white) enrolled in NAS in Greater Boston, MA area (1991-1997)	PP	Blood Pb: Overall mean (SD): 6.12 (4.03) $\mu\text{g/dL}$ Quintile means (SD): Q1: 2.3 (0.8) $\mu\text{g/dL}$ Q2: 3.9 (0.3) $\mu\text{g/dL}$ Q3: 5.4 (0.5) $\mu\text{g/dL}$ Q4: 7.4 (0.6) $\mu\text{g/dL}$ Q5: 12.4 (4.4) $\mu\text{g/dL}$ Tibia Pb: Overall median: 19 $\mu\text{g/g}$ Quintile means (SD): Q1: 7.4 (3.2) $\mu\text{g/g}$ Q2: 14.1 (1.4) $\mu\text{g/g}$ Q3: 18.9 (1.4) $\mu\text{g/g}$ Q4: 24.9 (2.2) $\mu\text{g/g}$ Q5: 40.9 (14) $\mu\text{g/g}$	BP association assessed using Spearman correlation coefficients. PP association (adjusted mean difference) assessed using multiple linear regression model adjusted for age, height, race, heart rate, waist circumference, diabetes, family history of hypertension, education level achieved, smoking, alcohol intake, fasting plasma glucose, and ratio of total cholesterol to HDL cholesterol	PP 4.2 (1.9, 6.5) mmHg higher in men with tibia Pb >19 $\mu\text{g/g}$ (median) compared with men with tibia Pb below the median Blood Pb (mean difference): Q5: -1.49 (-4.93, 1.94) Q4: -1.39 (-4.94, 2.15), Q3: -2.56 (-5.78, 0.67) Q2: -4.37 (-7.88, -0.86) Q1: Referent group Tibia Pb (mean difference): Q5: 2.58 (-1.15, 6.33) Q4: 2.64 (-0.93, 6.21) Q3: -0.73 (-4.27, 2.82) Q2: -3.02 (-6.48, 0.44) Q1: Referent group

Table 4-18 (Continued): Additional characteristics and quantitative data for associations of blood and bone Pb with BP measures for studies presented in Figure 4-17.

Study	Study Population / Methodology	Parameter	Pb Data	Statistical Analysis	Effect Estimate β (95% CI)
Peters et al. (2007)	Longitudinal and Cross-sectional 513 elderly men (mostly white, mean age 67 yr) enrolled in NAS in Greater Boston, MA area	BP	Tibia Pb: mean (SD): 21.5 (13.4) $\mu\text{g/g}$ Patella Pb: Mean (SD): 31.5 (19.3) $\mu\text{g/g}$	Logistic and linear regression models adjusted for age, age squared, sodium, potassium, and Ca^{2+} intake, family history of hypertension, BMI, educational level, pack-years of smoking, alcohol consumption, and physical activity	SBP Tibia Pb/ High Stress: 3.57 (0.39, 6.75) Tibia Pb/ Low Stress: 0.21 (-1.70, 1.29) per SD increase in tibia Pb Patella Pb/ High Stress: 2.98 (-0.12, 6.08) per SD increase in patella Pb Patella Pb/ Low Stress: NR
Elmarsafawy et al. (2006)	Cross-sectional 471 elderly men (mostly white, mean age 67 yr) enrolled in NAS in Greater Boston, MA area	BP	Blood Pb: Mean (SD): 6.6 (4.3) $\mu\text{g/dL}$ Tibia Pb: Mean (SD): 21.6 (12.0) $\mu\text{g/g}$ Patella Pb: Mean (SD): 31.7 (18.3) $\mu\text{g/g}$	Linear regression models adjusted for age, BMI, family history of hypertension, history of smoking, dietary sodium intake, and cumulative alcohol ingestion Lack of consideration for potential confounding by SES-related variables.	SBP Tibia Pb High Ca^{2+} group (>800 mg/day): 0.40 (0.11, 0.70) Low Ca^{2+} group (<800 mg/day): 0.19 (0.01, 0.37) mmHg per $\mu\text{g/g}$ tibia Pb

Table 4-18 (Continued): Additional characteristics and quantitative data for associations of blood and bone Pb with BP measures for studies presented in Figure 4-17.

Study	Study Population / Methodology	Parameter	Pb Data	Statistical Analysis	Effect Estimate β (95% CI)
Zhang et al. (2010a)	Cross-sectional 619 elderly men (mostly white, mean age 67 yr) enrolled in the NAS in Greater Boston, MA area	PP	Wild type HFE Tibia Pb: Median (IQR): 8 (12-27) $\mu\text{g/g}$ Patella Pb: Median (IQR): 26 (17-37) $\mu\text{g/g}$ C282Y HFE Tibia Pb: Median (IQR): 20 (14-27) $\mu\text{g/g}$ Patella Pb: Median (IQR): 25 (17-37) $\mu\text{g/g}$ H63D HFE Tibia Pb: Median (IQR): 19 (14-26) $\mu\text{g/g}$ Patella Pb: Median (IQR): 27 (19-37) $\mu\text{g/g}$	Linear mixed effects regression models with repeated measurements adjusted for age; education; alcohol intake; smoking; daily intakes of Ca^{2+} , sodium, and potassium; total calories; family history of hypertension; diabetes; height; heart rate; high-density lipoprotein (HDL); total cholesterol:HDL ratio; and waist circumference	PP mmHg per 13 $\mu\text{g/g}$ Tibia Pb: Wild Type HFE: 0.38 (0,1.96) H63D HFE: 3.30 (0.16, 6.46) C282Y HFE: 0.89 (0, 5.24) Any HFE variant: 2.90 (0.31, 5.51) mmHg per 19 $\mu\text{g/g}$ Patella Pb: Wild Type HFE: 0.26 (0, 1.78) H63D HFE: 2.95 (0, 5.92) C282Y HFE: 0.55 (0, 1.66) Any HFE variant: 2.83 (0.32,5.37)

Table 4-18 (Continued): Additional characteristics and quantitative data for associations of blood and bone Pb with BP measures for studies presented in Figure 4-17.

Study	Study Population / Methodology	Parameter	Pb Data	Statistical Analysis	Effect Estimate β (95% CI)
Scinicariello et al. (2010)	Cross-sectional 6,016 NHANES III (1988-1994) participants age $\geq$ 17 yr	BP	Concurrent Blood Pb: Mean (SE): Overall: 2.99 (0.09) $\mu\text{g/dL}$ Non-Hispanic Whites: 2.87 (0.09) Non-Hispanic Blacks 3.59 (0.20) Mexican American 3.33 (0.11)	Multivariable linear regression of log-transformed blood Pb level adjusted for age, sex, education, smoking status, alcohol intake, BMI, serum creatinine levels, serum Ca^{2+} , glycosylated hemoglobin, and hematocrit	SBP Non-Hispanic whites: 1.05 (0.32, 1.78) Non-Hispanic blacks: 2.55 (1.59, 3.51) Mexican Americans: 0.84 (-0.06, 1.74) DBP Non-Hispanic whites: -0.14 (-1.1, 0.82) Non-Hispanic blacks: 1.99 (1.13, 2.85) Mexican Americans: 0.74 (-0.005, 1.48) mmHg per unit increase in logarithmic (ln)-transformed blood Pb Significant interactions with blood Pb and ALAD genotype observed in relation to SBP for non-Hispanic whites and non-Hispanic blacks

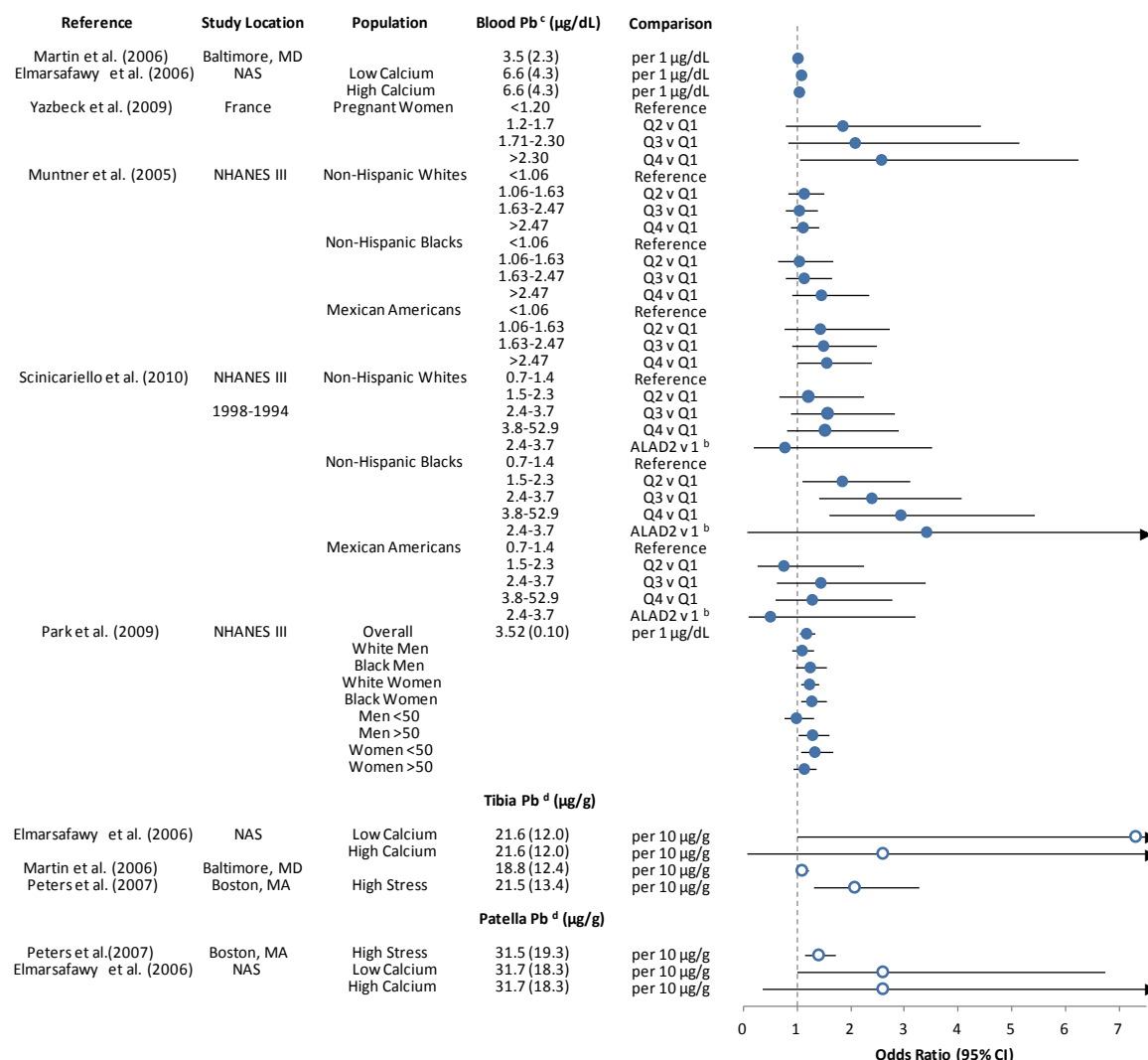
Table 4-18 (Continued): Additional characteristics and quantitative data for associations of blood and bone Pb with BP measures for studies presented in Figure 4-17.

Study	Study Population / Methodology	Parameter	Pb Data	Statistical Analysis	Effect Estimate β (95% CI)
Navas-Acien et al. (2008) ^b	Longitudinal and Cross-sectional Meta-analysis of studies using bone Pb as an exposure metric and BP as the outcome (8 studies)	BP	-	Inverse variance weighted random-effects meta-analyses	BP Pooled Estimates mmHg per 10 $\mu\text{g/g}$ Tibia Pb Prospective SBP 0.33 (-0.44, 1.11) Cross-sectional SBP 0.26 (0.02, 0.50) Cross-sectional DBP 0.02 (-0.15, 0.19) Hypertension per 10 $\mu\text{g/g}$ patella Pb Cross-sectional hypertension OR: 1.04 (1.01, 1.07) Meta-analysis estimate hypertension OR: 1.04 (0.96, 1.12)
Yazbeck et al. (2009) ^c	Cross-sectional 971 pregnant women, ages 18-45 yr, in France	BP	Midpregnancy Blood Pb: PIH group mean (SD): 2.2 (1.4) No PIH group mean (SD): 1.9 (1.2)	Multivariable logistic regression models adjusted for maternal age; Cd, Mn, and Se blood levels; hematocrit; parity; BMI; pregnancy weight gain; gestational diabetes; educational level; SES; geographic residence; and smoking status and alcohol consumption before and during pregnancy	Log-transformed blood Pb at mid-pregnancy SBP: $r = 0.08$; $p = 0.03$ DBP: $r = 0.07$; $p = 0.03$ Significant correlations also observed after 24 weeks of gestation and after 36 weeks of gestation.

^a95% CIs estimated from given p-value.

^bReference not included in [Figure 4-17](#), because it is a meta-analysis.

^cReference not included in [Figure 4-17](#), because only correlations were reported



Note: Studies are categorized by Pb biomarker (blood Pb [closed circles] or bone Pb [open circles]) for hypertension prevalence and incidence^a. Within each category, studies generally are presented in order of discussion in the text.

^aThe outcomes plotted are hypertension prevalence with the exception of Yazbeck et al. (2009) which measured pregnancy induced hypertension and Peters et al. (2007) which measured hypertension incidence.

^bb: ALAD2 vs. 1 indicates comparison between ALAD 2 carriers (e.g., ALAD1-2 and ALAD2-2) and ALAD 1 homozygotes (e.g., ALAD1-1).

^cc: Effect estimates were standardized to a 1 µg/dL increase in blood Pb (closed circles).

^dd: Effect estimates were standardized to a 10 µg/g increase in bone Pb (open circles).

Figure 4-18 Odds ratios (95% CI) for associations of blood Pb and bone Pb with hypertension prevalence and incidence.

Table 4-19 Additional characteristics and quantitative data for results presented in Figure 4-18 for associations of blood and bone Pb with hypertension measures.

Study (Ordered as they appear in the text)	Study Population and Methodology	Parameter	Pb Data	Statistical Analysis	Effect Estimate (95% CI)
Martin et al. (2006)	Cross-sectional 964 men and women, ages 50-70 yr, 40% African American, 55% White, 5% other, in Baltimore, MD	Hypertension (current use of antihypertensive medication, mean SBP $\geq$ 140 mmHg or DBP $\geq$ 90 mmHg)	Blood Pb: Mean (SD): 3.5 (2.3) $\mu\text{g/dL}$ Tibia Pb: Mean (SD): 18.8 (12.4) $\mu\text{g/g}$	Logistic regression models adjusted for age, sex, BMI, antihypertensive medication use, dietary sodium intake, dietary potassium intake, time of day, testing technician, and serum homocysteine Lack of consideration for potential confounding by SES-related variables.	Blood Pb level: OR=1.02 (0.87, 1.19) Tibia Pb: OR=1.24 (1.05, 1.47)
Weaver et al. (2008) ^a	Cross-sectional 652 current and former Pb workers in South Korea (12/1999-6/2001)	Hypertension (mean SBP $\geq$ 140 mmHg, DBP $\geq$ 90 mmHg; and/or use of antihypertensive medications; or physician diagnosis)	Blood Pb: Mean (SD): 31.9 (14.8) $\mu\text{g/dL}$ Patella Pb: Mean (SD): 37.5 (41.8) $\mu\text{g/g}$	Logistic regression models adjusted for age, sex, BMI, diabetes, antihypertensive and analgesic medication use, Pb job duration, work status, tobacco and alcohol use	Quantitative results not reported. None of the examined Pb exposure metrics (blood, patella, and logarithmic (ln)-transformed patella) were significantly associated with hypertension
Peters et al. (2007)	Longitudinal 513 elderly men (mostly white, mean age 67 yr) enrolled in NAS in Greater Boston, MA area	Hypertension (mean SBP $>$ 140 mmHg, DBP $>$ 90 mmHg; or physician diagnosis)	Tibia Pb: Mean (SD): 21.5 (13.4) $\mu\text{g/g}$ Patella Pb: Mean (SD): 31.5 (19.3) $\mu\text{g/g}$	Cox proportional hazards models adjusted for age, age squared, sodium, potassium, and Ca^{2+} intake, family history of hypertension, BMI, educational level, smoking, alcohol consumption, baseline SBP and DBP, and physical activity	Risk of Hypertension Incidence High Stress RR=2.66 (1.43, 4.95) per SD increase in tibia Pb RR=2.64 (1.42, 4.92) per SD increase in patella Pb

Table 4-19 (Continued): Additional characteristics and quantitative data for results presented in Figure 4-18 for associations of blood and bone Pb with hypertension measures.

Study (Ordered as they appear in the text)	Study Population and Methodology	Parameter	Pb Data	Statistical Analysis	Effect Estimate (95% CI)
Elmarsafawy et al. (2006)	Cross-sectional 471 elderly men (mostly white, mean age 67 yr) enrolled in NAS in Greater Boston, MA area	Hypertension (mean SBP $\geq$ 160 mmHg, DBP $\geq$ 95 mmHg; and/or physician diagnosis with current use of antihypertensive medications)	Blood Pb: Mean (SD): 6.6 (4.3) $\mu\text{g/dL}$ Tibia Pb: Mean (SD): 21.6 (12.0) $\mu\text{g/g}$ Patella Pb: Mean (SD): 31.7 (18.3) $\mu\text{g/g}$	Logistic regression models adjusted for age, BMI, family history of hypertension, history of smoking, dietary sodium intake, and cumulative alcohol ingestion	Low Ca^{2+} group (<800 mg/day): Blood Pb OR: 1.07 (1.00, 1.15) Tibia Pb OR: 1.02 (1.00, 1.04) Patella Pb OR: 1.01 (1.00, 1.03) High Ca^{2+} group (>800 mg/day): Blood Pb OR: 1.03 (0.97, 1.11) Tibia Pb OR: 1.01 (0.97, 1.04) Patella Pb OR: 1.01 (0.99, 1.03) Per $\mu\text{g/dL}$ blood Pb or $\mu\text{g/g}$ tibia or patella Pb
Yazbeck et al. (2009) ^a	Cross-sectional 971 pregnant women, ages 18-45 yr, in France	PIH (SBP $\geq$ 140 mmHg or DBP $\geq$ 90 mmHg after the 22nd week of gestation)	Blood Pb ($\mu\text{g/dL}$) PIH group mean (SD): 2.2 (1.4) No PIH group mean (SD): 1.9 (1.2) Q1: <1.20 Q2: 1.20-1.70 Q3: 1.71-2.30 Q4: >2.30	Multivariable logistic regression models adjusted for maternal age, Cd, Mn, and Se blood levels, parity, hematocrit, BMI, gestational diabetes, educational levels, SES, geographic residence, and smoking status during pregnancy	PIH Blood Pb OR=3.29 (1.11, 9.74) per 1 unit increase in log maternal blood Pb level Q1: Reference group Q2: OR=1.84 (0.77, 4.41) Q3: OR=2.07 (0.83, 5.13) Q4: OR=2.56 (1.05, 6.22)

Table 4-19 (Continued): Additional characteristics and quantitative data for results presented in Figure 4-18 for associations of blood and bone Pb with hypertension measures.

Study (Ordered as they appear in the text)	Study Population and Methodology	Parameter	Pb Data	Statistical Analysis	Effect Estimate (95% CI)
Muntner et al. (2005)	Cross-sectional 9,961 NHANES (1999-2002) participants, ages ≥18 yr	Hypertension (current use of antihypertensive medication, SBP ≥ 140 mmHg, or DBP ≥ 90 mmHg)	Concurrent Blood Pb (µg/dL) Overall Mean (95% CI): 1.64 (1.59-1.68) Q1: <1.06 Q2: 1.06-1.63 Q3: 1.63-2.47 Q4: ≥ 2.47	Multivariable logistic regression models adjusted for age, sex, diabetes mellitus, BMI, cigarette smoking, alcohol consumption, high school education, and health insurance status Monotonic increase in OR across blood Pb level groups.	Non-Hispanic white: Q1: Reference group Q2: OR=1.12 (0.83, 1.50) Q3: OR=1.03 (0.78, 1.37) Q4: OR=1.10 (0.87, 1.41) Non-Hispanic black Q1: Reference group Q2: OR=1.03 (0.63, 1.67) Q3: OR=1.12 (0.77, 1.64) Q4: OR=1.44 (0.89, 2.32) Mexican American Q1: Reference group Q2: OR=1.42 (0.75, 2.71) Q2: OR=1.48 (0.89, 2.48) Q3: OR=1.54 (0.99, 2.39) p for trend=0.04

Table 4-19 (Continued): Additional characteristics and quantitative data for results presented in Figure 4-18 for associations of blood and bone Pb with hypertension measures.

Study (Ordered as they appear in the text)	Study Population and Methodology	Parameter	Pb Data	Statistical Analysis	Effect Estimate (95% CI)
Scinicariello et al. (2010)	Cross-sectional 6,016 NHANES III (1988-1994) participants, ages ≥ 17 yr	Hypertension (current use of antihypertensive medication, SBP ≥ 140 mmHg, or DBP ≥ 90 mmHg)	Concurrent Blood Pb (µg/dL) Mean (SE): 2.99 (0.09) Q1: 0.7-1.4 Q2: 1.5-2.3 Q3: 2.4-3.7 Q4: 3.8-52.9 Mean (SE): Non-Hispanic Whites: 2.87 (0.09) Non-Hispanic Blacks: 3.59 (0.20) Mexican American: 3.33 (0.11)	Multivariable logistic regression model adjusted for race/ethnicity, age, sex, education, smoking status, alcohol intake, BMI, serum creatinine levels, serum Ca ²⁺ , glycosylated hemoglobin, and hematocrit	Non-Hispanic whites: Q1: Reference group Q2: POR=1.21 (0.66, 2.24) Q3: POR=1.57 (0.88, 2.80) Q4: POR=1.52 (0.80, 2.88) ALAD1-2/2-2: POR= 0.76 (0.17, 3.50) ALAD-1: Reference group Non-Hispanic blacks: Q1: Reference Q2: POR=1.83 (1.08, 3.09) Q3: POR=2.38 (1.40, 4.06) Q4: POR=2.92 (1.58, 5.41) ALAD1-2/2-2: POR= 3.40 (0.05, 219.03) ALAD-1: Reference group Mexican Americans: Q1: Reference Q2: POR=0.74 (0.24, 2.23) Q3: POR=1.43 (0.61, 3.38) Q4: POR=1.27 (0.59, 2.75) ALAD1-2/2-2: POR= 0.49 (0.08, 3.20) ALAD-1: Reference group ALAD2 carriers in non-Hispanic white population in highest blood Pb quartile with blood Pb level <10 µg/dL associated with hypertension compared to ALAD1 homozygous individuals (POR=1.86 [1.00, 3.49]).

Table 4-19 (Continued): Additional characteristics and quantitative data for results presented in Figure 4-18 for associations of blood and bone Pb with hypertension measures.

Study (Ordered as they appear in the text)	Study Population and Methodology	Parameter	Pb Data	Statistical Analysis	Effect Estimate (95% CI)
Park et al. (2009b)	Cross-sectional 12,500 NHANES III (1988-1994) participants, mean age 49.9 yr	Hypertension	Concurrent Blood Pb Mean and SE (µg/dL) Overall: 3.52 (0.10) White men <50 yr: 4.02 (0.16) ≥ 50 yr: 4.92 (0.18) Black men <50 yr: 4.55 (0.15) ≥ 50 yr: 7.57 (0.22) White women <50 yr: 2.09 (0.07) ≥ 50 yr: 3.53 (0.12) Black women <50 yr: 2.52 (0.09) ≥ 50 yr: 4.49 (0.16)	Logistic regression models adjusted for age, education, smoking status, cigarette smoking, BMI, hematocrit, alcohol consumption, physical activity, antihypertensive medication use, and diagnosis of type-2 diabetes	OR per SD (0.75 µg/dL) in log blood Pb: Overall: 1.12 (1.03, 1.23). White men: 1.06 (0.92, 1.22) Black men: 1.17 (0.98, 1.38) White women: 1.16 (1.04, 1.29) Black women: 1.19 (1.04, 1.38) Men <50 yr: 0.98 (0.80, 1.22) Men >50 yr: 1.20 (1.02, 1.41) Women <50 yr: 1.23 (1.04, 1.46) Women >50 yr: 1.09 (0.94, 1.26)

^aNot included in [Figure 4-18](#) because OR data were not reported.

In an occupational cohort in South Korea, Glenn et al. (2006) simultaneously modeled multiple Pb dose measures of individuals collected repeatedly over four years of follow up. Thus, through the assessment of cross-sectional and longitudinal relationships with BP, this study provided key insight on potentially important time periods of Pb exposure and also informed the directionality of association. The initial blood Pb level was used as a baseline covariate and the difference in blood Pb level between visits was computed for each subsequent visit. The bone Pb measures (tibia Pb at visits 1 and 2, patella Pb at visit 3) were used to indicate historical exposure and cumulative dose. Four models were specified: Model 1 was conceptualized to reflect short-term changes in BP associated with recent dose; Model 2 to reflect longer-term changes associated with cumulative dose controlling for the association of baseline BP with recent dose; Model 3 to reflect longer-term changes associated with cumulative dose controlling for cross-sectional influence of cumulative dose on baseline BP; and Model 4 to reflect both short-term change with recent dose and longer-term change with cumulative dose. Concurrent blood Pb and increases in blood Pb between visits were associated with increases in systolic BP in Model 1 (short-term dose) and Model 4 (short- and longer-term dose). No association was observed between BP and tibia Pb at baseline while higher tibia Pb was associated with a decrease in systolic BP in each of the models.

Glenn et al. (2006) was strengthened by the analysis of associations between changes in blood Pb and changes in BP over time within individual subjects. These results indicate that circulating Pb (e.g., blood Pb) may act continuously on systolic BP and reductions in blood Pb may contribute to reductions in BP, while cumulative Pb exposure (represented by bone Pb in this study) may contribute to hypertension incidence by different mechanisms over longer time periods and in older subjects. This analysis in subjects (mean [SD] age at baseline 41.4 [9.5] years) with a low prevalence of hypertension suggests at least one of the biological pathways that influences how systolic BP responds to Pb operates over a relatively rapid timeframe. This may reflect an immediate response to Pb at a biochemical site of action as a consequence of the biologically available Pb circulating in blood. A persistent effect of cumulative doses over a lifetime may occur via other mechanisms. Bone Pb level may exert influence on blood Pb levels and consequently on BP in an aging population with prolonged Pb exposure. Thus, the findings contribute important information regarding the various short and long-term exposure relationships with increases in BP and hypertension. It is important to acknowledge the uncertainty regarding the applicability of these findings regarding short-term and long-term effects in Pb workers with relatively high current Pb exposures contributing to blood Pb levels (mean blood Pb levels over time: 20-37 µg/dL) to adults in the U.S. general population whose concurrent blood Pb levels are influenced more by Pb mobilized from bone stores. Further, for bone Pb analysis, the potential for bone Pb, BP, and hypertension findings to be impacted by residual confounding by age may be a

factor to consider in older populations since exposure studies of older cohorts (NAS/ mean age >60 years; ([Wilker et al., 2011](#); [Kim et al., 1997](#))) indicate that bone Pb is correlated with age.

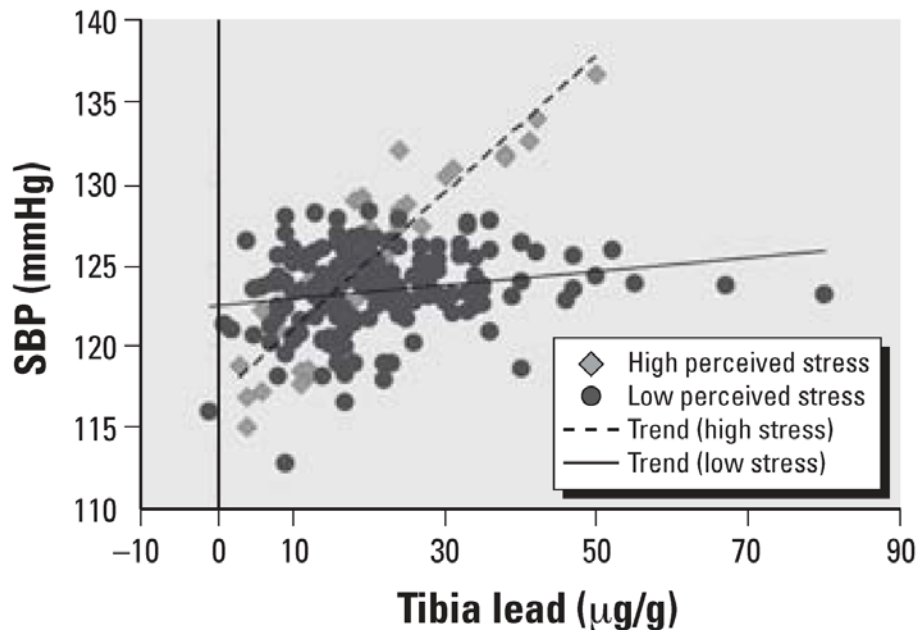
In a separate cross-sectional analysis of the same occupationally-exposed group in year three of follow-up, Weaver et al. ([2008](#)) examined associations of concurrent patella Pb and blood Pb level with systolic BP, diastolic BP, and hypertension and effect modification by ALAD (the genotypes have different affinities for Pb) and vitamin D receptor (VDR) polymorphisms. None of the Pb biomarkers were associated with diastolic BP. Patella Pb alone was not significantly associated with systolic BP. However, blood Pb, either alone or with patella Pb, was significantly associated with higher systolic BP. The patella Pb-age and blood Pb-age interactions were not statistically significant. There were no significant associations of blood Pb or patella Pb with hypertension status or effect modification by age or sex. Further, interactions between polymorphisms of the VDR and of ALAD variants with blood Pb and patella Pb on systolic BP were not statistically significant. Mean blood Pb level was high (30.9 µg/dL) compared to non-occupational groups.

Weaver et al. ([2010](#)) provided the results of further analysis of this Korean worker cohort, with a focus on determining the functional form of the concentration-response relationships. In a log linear model, the coefficient indicated that every doubling of blood Pb level was associated with a systolic BP increase of 1.76 mmHg. The J test, a statistical test for determining which, if either, of two functional forms of the same variable provides a superior fit to data in non-nested models ([Davidson and MacKinnon, 1981](#)), returned a p-value of 0.013 in favor of the natural log blood Pb level over the linear blood Pb level specification. This analysis indicates that the systolic BP increase in this cohort is better described as a logarithmic function of blood Pb level within the range of the study than by a linear function.

Several analyses in the NAS cohort of predominantly white older men in the greater Boston area found associations of blood and bone Pb level with BP and hypertension, and they indicated effect modification by calcium intake, perceived stress, and HFE gene variants. In a cross-sectional analysis, Perlstein et al. ([2007](#)) found a statistically significant association between blood Pb and diastolic BP in adjusted models. The subjects in this study had at least one bone Pb measurement during the years 1991-1997 and were not on antihypertensive medication at the time of the measurement. While tibia Pb was not significantly associated with BP, it was associated with pulse pressure (PP). Men with tibia Pb above the median (19 µg/g) had a higher mean PP (4.2 mmHg [95% CI: 1.9, 6.5]) compared to men with tibia Pb below the median. The trend toward increasing PP with increasing quintile of tibia Pb was statistically significant although

none of the confidence intervals for PP referenced to the lowest quintile of tibia Pb (<7.4 µg/g) excluded the null value.

Peters et al. (2007) examined cross-sectionally the modification of the associations of tibia and patella Pb with BP and hypertension by self-reported stress (assessed by questionnaire) in NAS men. High stress also has been linked with higher BP, potentially via mechanisms also affected by Pb exposure including activation of sympathetic pathways, ROS, and the HPA axis. Among all subjects, higher bone Pb level was associated (statistically nonsignificant) with greater odds of hypertension status and higher systolic BP. As indicated in Figure 4-19, the association between systolic BP and tibia Pb differed between those with high and low self-reported stress (β for tibia Pb x stress interaction = 3.77 mmHg [95% CI: 0.46, 7.09]) per SD increase in tibia Pb. Stress also was found to modify the patella Pb-BP association (β for patella Pb x stress interaction = 2.60 mmHg [95% CI: -0.95, 6.15] per SD increase in patella Pb). Neither bone, self-reported stress, nor their interaction was associated significantly with diastolic BP. Peters et al. (2007) also used Cox proportional hazards models to assess the interaction of stress and bone Pb level in the development of hypertension among those free of hypertension at baseline. The results of this analysis showed that increasing tibia and patella Pb were associated with greater risk of developing hypertension among those with high stress compared to those with lower perceived stress (RR of developing hypertension among those with high stress: 2.66 [95% CI: 1.43, 4.95] per SD increase in tibia Pb and 2.64 [95% CI: 1.42, 4.92] per SD increase in patella Pb). These results provide evidence supporting adults with higher stress as a population potentially at increased risk of Pb-associated cardiovascular effects. Earlier, Cheng et al. (2001) examined the NAS cohort in 474 subjects without hypertension (mean [SD] blood Pb level: 5.87 [4.01]) at baseline measurement and analyzed linear models with patella Pb and reported that only patella Pb level was associated with a significant increase in the rate ratio for hypertension using a Cox's proportional hazards model.



Source: Peters et al. (2007)

Figure 4-19 The relationship between tibia Pb and estimated systolic BP (SBP) for those with high self-reported stress versus those with low self-reported stress.

Elmarsafawy et al. (2006) examined the modification of the relationship between Pb and hypertension by dietary calcium, with 467 subjects from the NAS. Responses on a semi-quantitative dietary frequency questionnaire with one-year recall were used to estimate calcium intake. Effect modification by calcium intake (dichotomized at 800 mg/day) was examined using interaction terms in logistic regression models and by conducting analyses stratified on the calcium variable. Increasing bone and blood Pb increased the odds of hypertension, particularly among subjects with low dietary calcium (i.e., <800 mg/day).

Zhang et al. (2010a) examined the effect of polymorphisms of the hemochromatosis gene (HFE) on the relationship of bone Pb with PP in NAS men. HFE polymorphisms promote Fe absorption and have been shown to modify the impact of adult cardiac function. The H63D variant is associated with hemochromatosis, a disease characterized by higher Fe body burden. Subjects had up to three PP measurements during the 10 year study period. The overall results demonstrated a strong relationship between bone Pb and PP in this study, similar to an earlier cross-sectional PP study of many of the same subjects (Perlstein et al., 2007). Zhang et al. (2010a) extended these findings by demonstrating

larger increases in PP per unit increase in tibia and patella Pb level among those with the H63D variant compared to those with the wild-type or the C282Y variant.

A small number of cross-sectional studies examined and found that blood Pb level was associated with hypertension in pregnancy. Yazbeck et al. ([2009](#)) examined a community-based group of pregnant women in France and unlike most other studies, adjusted for potential confounding by blood concentrations of Cd, Mn, and Se. Pregnancy induced hypertension (PIH) was defined as systolic BP >140 mmHg and/or diastolic BP >90 mmHg during at least two clinic visits after week 22 of gestation. Patients with pre-existing chronic hypertension were excluded. The mean (SD) blood Pb levels measured during pregnancy were 2.2 (1.4) µg/dL in PIH cases and 1.9 (1.2) µg/dL in normotensive women. An association between blood Pb and PIH was observed (OR 3.29 [95% CI: 1.11, 9.74] per unit increase in log-transformed blood Pb level). Cd and Se concentrations were comparable between PIH and no PIH groups. Adjustment for the metals slightly attenuated but did not eliminate the association between blood Pb levels and the risk of PIH. Investigators observed no significant interactions among blood Pb level, any of the other elements, and maternal characteristics in predicting the risk of PIH. Interaction between blood Se and Pb concentrations was not significant, and the putative protection effects of Se through antioxidative properties were not found in this study.

Wells et al. ([2011b](#)) measured the relationship of cord blood Pb with BP in 285 women at admission to the Johns Hopkins Hospital in Baltimore, MD, during labor and delivery. Women with cord blood Pb levels in the highest quartile for the study group (>0.96 µg/dL) had significantly higher systolic and diastolic BP (upon admission and for maximum BP) compared to women in the first quartile (<0.46 µg/dL). The level of uncertainty at these levels of exposure is difficult to estimate. The authors used Benchmark Dose Software V2.1, developed by the EPA, to estimate the blood Pb level (benchmark dose or BMD) and the associated lower confidence limit (BMDL) that was associated with one SD increase in BP. In this study group, one SD is approximately equivalent to a 10% increase above the mean for the first quartile blood Pb reference group. The BMD approach was used only as a means of quantifying the relationship of blood Pb with BP in this population. This analysis indicated that the BMDL for the maternal blood Pb level (estimated from cord blood Pb levels) that was associated with a 1 SD increase in all BP outcomes was about 1.4 µg/dL. These reported results are similar to those reported in the 2006 Pb AQCD as well as those found 25 years ago but with blood Pb levels an order of magnitude lower in the more recent study. However, uncertainty exists as to the specific Pb exposure level, timing, frequency, and duration that contributed to the observed associations.

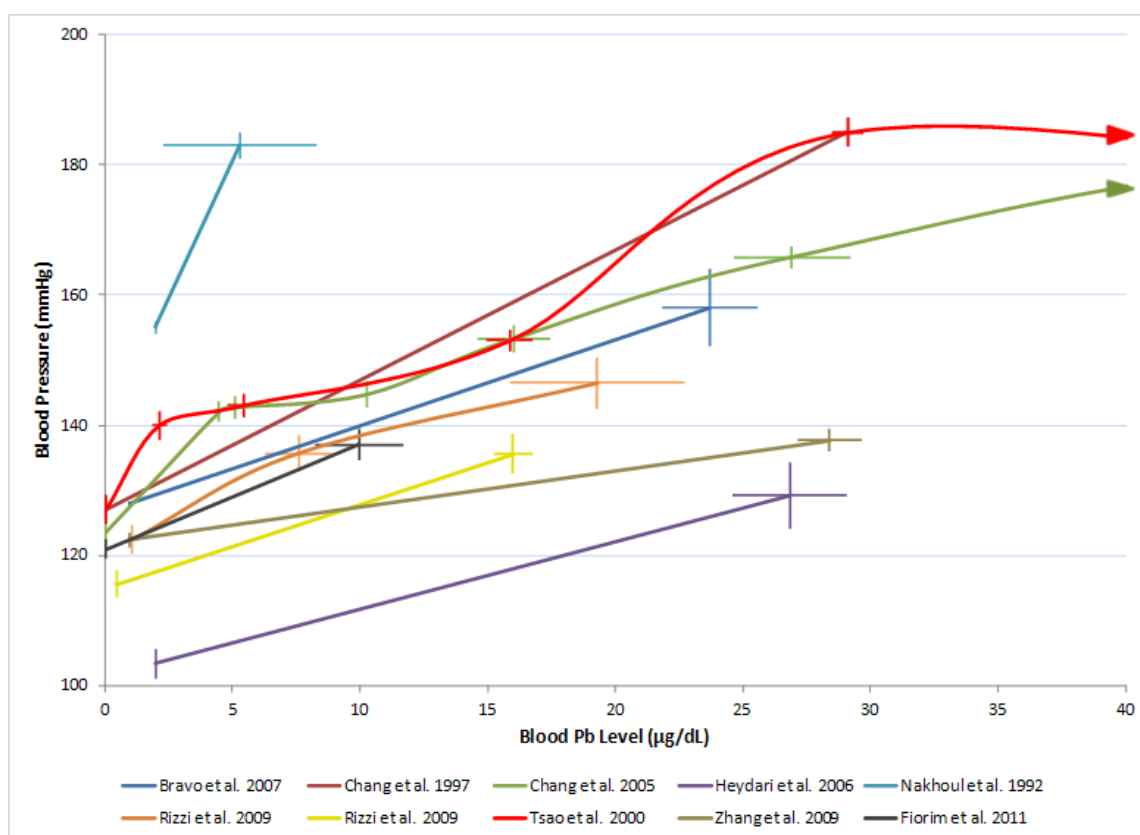
Recent analyses using NHANES data continued to indicate associations of Pb biomarkers with BP and hypertension. Muntner et al. (2005) previously used the NHANES 1999-2002 data to indicate that concurrent blood Pb levels were associated with hypertension, peripheral artery disease (PAD), and chronic kidney disease. The PAD results are discussed later in Section 4.4.3.5, and chronic kidney disease results are discussed in Section 4.5.2.1. Blood Pb increased regularly with age (geometric means [95% CIs]: 1.28 µg/dL [1.23, 1.33] in the 18-39 year age group to 2.32 µg/dL [2.20, 2.44] in the 75 year and older age group). Associations were observed between concurrent blood Pb level and hypertension across race/ethnicity groups with significant trends observed for non-Hispanic blacks and Mexican Americans.

In the NHANES III 1988-1994 population, Scinicariello et al. (2010) found a gene-environment interaction between blood Pb level and ALAD genotype (the genotypes have different affinities for Pb) in relation to systolic BP and diastolic BP in a cross-sectional analysis. These interactions varied across race/ethnicity strata. The strongest associations were observed among non-Hispanic blacks (Figure 4-17, Table 4-18). A statistically significant interaction was observed between concurrent blood Pb level and ALAD1-2/2-2b (i.e., ALAD2 carriers) among non-Hispanic whites and non-Hispanic blacks. Scinicariello et al. (2010) also found an interaction between ALAD genotype and blood Pb level in the association with hypertension. Statistically significant associations between concurrent blood Pb level and hypertension were observed among non-Hispanic blacks and nonsignificant increases were observed among non-Hispanic whites and Mexican Americans (with the exception of Mexican Americans in the second quartile of blood Pb level) (Figure 4-18, Table 4-19). In addition, non-Hispanic white ALAD2 carriers in the highest blood Pb level quartile 3.8-52.9 µg/dL) had a significantly higher association with hypertension compared with ALAD1 homozygous individuals in the highest quartile of blood Pb. In the same NHANES population, Park et al. (2009b) predicted bone Pb levels using a model developed with NAS data. Concurrent blood Pb was associated with hypertension overall in the NHANES population, with larger associations observed among black men and women as well as older adults (Figure 4-18, Table 4-19). Associations also were observed with estimated bone Pb.

4.4.2.2 Toxicology

Studies on the effect of Pb (as blood Pb level) on systolic BP in unanesthetized adult rats consistently reported an increase in BP with increasing blood Pb level as shown in Figure 4-20 (results summarized in Table 4-20). An array of studies has provided evidence that long-term Pb exposure (>4 weeks), resulting in blood Pb levels less than 10 µg/dL, which is relevant to humans in the U.S., can result in the onset of hypertension (after a latency

period) in experimental animals that persists long after the cessation of Pb exposure ([U.S. EPA, 2006b](#)). Tsao et al. (2000) presented evidence for increased systolic and diastolic BP in rats with blood Pb levels somewhat similar to the current U.S. adult population (mean [SD]: 2.15 [0.92] $\mu\text{g/dL}$ blood Pb; 140 [7] mmHg systolic BP, 98 [7] mmHg diastolic BP) compared to untreated controls (mean [SD]: 0.05 [0.05] $\mu\text{g/dL}$ blood Pb; 127 [7] mmHg systolic BP, 88 [7] mmHg diastolic BP). As this was the lowest Pb level tested, no evidence of a threshold was evident. Further, a test for linear trend revealed a statistically significant, positive trend for increasing BP with increasing blood Pb levels up to 56 $\mu\text{g/dL}$ (e.g., mean [SD]: 5.47 [2.1] $\mu\text{g/dL}$ blood Pb; 143 [6] mmHg systolic BP, 97 [8] mmHg diastolic BP), with the effect leveling off at higher blood Pb levels.



Note: Crosses represent standard error for blood Pb and BP measurements. If no crossbar is present, error results were not reported. Arrows represent higher doses tested.

Figure 4-20 Changes in BP after Pb exposure (represented as blood Pb level) in unanesthetized adult rats across studies.

Table 4-20 Characteristics of studies of blood Pb with BP measures in animals presented in Figure 4-20.

Reference ^a	Lifestage; Sex; Strain	Exposure Duration	Exposure Level; Route	Mean [SEM] ^b Blood Pb Level (µg/dL)	n	ΔSBP (mmHg; lowest blood Pb level compared with control) ^c	Comments
Fiorim et al. (2011)	Adult; M, Wistar Rat	7 days	4 µg/100 g followed by 0.05 µg/100 g daily; intramuscular	9.98 [1.7]	12	16	
Nakhoul et al. (1992)	Adult; M SH Rat	8 weeks	100 ppm; drinking water	5.3 [3]	7	28	Spontaneously hypertensive rat model
Chang et al. (2005)	Adult; M Wistar Rat	8 weeks	20,000 ppm then removal and measurements 1-7 mo after; drinking water	Range: 4.5 to 83	5	13.8	
Tsao et al. (2000)	Adult, M Wistar Rat	8 weeks	100 - 20,000 ppm; drinking water	Range of means: 2.15 [0.29] to 85.76 [1.29] ^b	10	13	
Rizzi et al. (2009)	Adult; M Wistar Rat	8 weeks	30, 90 ppm; drinking water	7.6 [1.3], 19.3 [3.4]	11	13.3	
Chang et al. (1997)	Adult; M Wistar Rat	8 weeks	500 ppm; drinking water	29.1 [0.6] ^b	10	58	
Heydari et al. (2006)	Adult; M Sprague- Dawley Rat	12 weeks	100 ppm; drinking water	26.8 [2.2]	6	25.8	
Bravo et al. (2007)	Adult; M Sprague- Dawley Rat	14 weeks	100 ppm; drinking water	23.7 [1.9] ^b	12	30	
Zhang et al. (2009a)	Adult; M Wistar Rat	40 weeks	100 ppm; drinking water	28.4 [1.1] ^b	8-10	15.3	

^aStudies are presented in order of increasing duration of exposure.

^bStandard deviation converted to SEM.

^cDifference in systolic BP (SBP) between group means not within one exposure group.

Experimental animal studies continued to provide evidence that long-term Pb exposure results in sustained arterial hypertension after a latency period. Systolic BP increased in rats after exposure to 90-10,000 ppm Pb (as Pb acetate in drinking water) for various time periods that resulted in blood Pb levels between 19.3-240 µg/dL ([Mohammad et al., 2010](#); [Zhang et al., 2009a](#); [Badavi et al., 2008](#); [Grizzo and Cordellini, 2008](#); [Reza et al., 2008](#); [Bravo et al., 2007](#); [Vargas-Robles et al., 2007](#); [Heydari et al., 2006](#); [Bagchi and Preuss, 2005](#)). Past studies have shown statistically significant elevations in BP in rats with lower blood Pb levels. For example, long-term Pb exposure to spontaneously hypertensive rats (resulting in mean [SEM] blood Pb level: 5.3 [3] µg/dL) led to increased BP ([Nakhoul et al., 1992](#)). Consistent with measurements of systolic BP by tail-cuff plethysmography, Pb exposure (100 ppm for 14 weeks; mean blood Pb level: 24 µg/dL) also caused an increase in intra-aortic mean arterial pressure ([Bravo et al., 2007](#)). In a study that tested low levels of Pb exposure (30 ppm; mean blood Pb level: 7.6 µg/dL), a statistically significant increase in systolic BP was not observed despite elevated blood Pb level after 8 weeks of treatment. Nonetheless, there was a trend of higher BP with increased blood Pb levels ([Rizzi et al., 2009](#)).

Studies found that Pb-induced increases in BP persisted long after cessation of Pb exposure. Bagchi and Preuss ([2005](#)) found that elevated systolic BP was maintained for 210 days after cessation of Pb exposure (10,000 ppm Pb acetate in water, 40 days, monitored for one year). However, chelation therapy using Na₂CaEDTA returned systolic BP to levels comparable to those in rats not treated with Pb ([Bagchi and Preuss, 2005](#)). Chang et al. ([2005](#)) reported a partial reversibility of effect after cessation of Pb exposure, where Pb-induced elevated BP decreased but did not return to control levels 7 months post Pb exposure. After Pb exposure was removed, blood, heart, aorta, and kidney Pb levels decreased quickly within the first three months ([Chang et al., 2005](#)). Pb-induced elevated systolic BP persisted for one month following Pb exposure cessation, followed by obvious decreases in BP until 4 months after Pb exposure cessation. Between 4 and 7 months after Pb exposure cessation, the still-elevated BP did not decrease further, thus never returning to control BP levels. Decreases in BP were strongly correlated with decreases in blood Pb level after exposure cessation.

The aforementioned studies all assessed the relationship between long-term exposure (>4 weeks) of rats to Pb and measures of BP. However, recent research also investigated BP elevation occurring after short-term treatment with Pb (<4 weeks). Studies found increased systolic BP after 7 days of Pb treatment (daily injections resulting in mean [SEM] blood Pb levels of 9.98 [1.7] µg/dL) ([Fiorim et al., 2011](#)) and after 2 weeks of Pb exposure (100 ppm via drinking water) ([Sharifi et al., 2004](#)). A study utilizing intra-arterial pressure measurements found that a single high-dose Pb injection in rats (resulting in mean [SEM] blood Pb levels of 37 [1.7] µg/dL) increased systolic arterial

pressure after only 60 minutes ([Simões et al., 2011](#)). The injection of Pb into the rat may not allow for extrapolation of these results to humans since this is not a comparable Pb exposure route. These studies suggest that there is the potential for an increase in BP following short-term Pb treatment. It is possible that the increases in BP following short- and long-term Pb exposures are occurring through separate mechanisms; however, studies using both short- and longer-term Pb exposure have correlated increased BP with an activation of the renin-angiotensin system (i.e., increase in angiotensin converting enzyme (ACE) activity) ([Section 4.4.2.3](#)). Several of these aforementioned studies used the injection route of Pb administration, and the relevance of these bolus doses over short periods of time to human routes of short-term exposure is uncertain. However, it is important to acknowledge that the results were similar to those from the study that examined short-term exposure to Pb via drinking water.

4.4.2.3 Hypertension Modes of Action

The 2006 Pb AQCD ([U.S. EPA, 2006b](#)) examined a number of mechanisms leading to Pb-induced hypertension, including oxidative stress, hormonal and blood pressure regulatory system dysfunction, vasomodulation, and cellular alterations. As described below, recent studies in experimental animals and cells further supported roles for these potential mechanisms in mediating hypertension from Pb exposure.

Oxidative Stress Response - Reactive Oxygen Species and Nitric Oxide

Several studies discussed in the 2006 Pb AQCD demonstrated a role for oxidative stress in the pathogenesis of Pb-induced hypertension, mediated by the inactivation of nitric oxide (NO) and downregulation of soluble guanylate cyclase (sGC) ([Dursun et al., 2005](#); [Attri et al., 2003](#); [Gonick et al., 1997](#); [Vaziri et al., 1997](#); [Khalil-Manesh et al., 1994](#); [Khalil-Manesh et al., 1993b](#)). Pb-induced reduction of biologically active NO was found not to be due to a reduction in NO-production capacity ([Vaziri and Ding, 2001](#); [Vaziri et al., 1999a](#)); instead, it was found to result from inactivation and sequestration of NO by ROS ([Malvezzi et al., 2001](#); [Vaziri et al., 1999b](#)). Oxidative stress from Pb exposure in animals may be due to upregulation of NAD(P)H oxidase ([Ni et al., 2004](#); [Vaziri et al., 2003](#)), induction of Fenton and Haber-Weiss reactions ([Ding et al., 2001](#); [Ding et al., 2000](#)), and failure of the antioxidant enzymes, CAT and GPx, to compensate for the increased ROS ([Farmand et al., 2005](#); [Vaziri et al., 2003](#)). Many biological actions of NO, such as vasorelaxation, are mediated by cGMP, which is produced by sGC from the substrate GTP. Oxidative stress also has been found to play a role in Pb-induced downregulation of sGC ([Farmand et al., 2005](#); [Courtois et al., 2003](#); [Marques et al.,](#)

[2001](#)). Thus, the reduction of the vasodilator NO from inactivation and sequestration by Pb-induced ROS leads to increased vasoconstriction and BP.

Pb-induced oxidative stress also has been found to induce renal tubulointerstitial inflammation which plays a crucial role in models of hypertension ([Rodriguez-Iturbe et al., 2005](#); [Rodriguez-Iturbe et al., 2004](#)). Tubulointerstitial inflammation from treatment with Pb has been coupled with activation of the redox sensitive NF- κ B ([Ramesh et al., 2001](#)). Pb-induced hypertension, inflammation, and NF- κ B activation can be ameliorated by antioxidant therapy ([Rodriguez-Iturbe et al., 2004](#)). There is mixed evidence to suggest that Pb-induced hypertension may also be promoted by activation of PKC leading to enhanced vascular contractility ([Valencia et al., 2001](#); [Watts et al., 1995](#)).

Recent studies continued to provide evidence for the role of ROS and NO metabolism in Pb-induced hypertension and vascular disease. Increased systolic BP after Pb exposure was accompanied by increased superoxide (O_2^-) and O_2^- -positive cells ([Bravo et al., 2007](#); [Vargas-Robles et al., 2007](#)), elevated urinary malondialdehyde (MDA, a measure of lipid peroxidation) ([Bravo et al., 2007](#)), and increased 3-nitrotyrosine ([Vargas-Robles et al., 2007](#)). Inhibition of NAD(P)H oxidase, an enzyme that generates O_2^- and hydrogen peroxide, was able to block Pb-induced (1 ppm) aortic contraction to 5-hydroxytryptamine (5-HT) ([Zhang et al., 2005](#)). Increases in systolic BP, intra-aortic mean arterial pressure, and MDA after Pb exposure (100 ppm; mean blood Pb level: 23.7 μ g/dL) were also prevented by treatment with the immunosuppressant, mycophenolate mofetil (MMF) (mean blood Pb level in MMF-treated animals: 27 μ g/dL) ([Bravo et al., 2007](#)). MMF has been shown to inhibit endothelial NAD(P)H oxidase, which could explain how it decreases Pb-induced increases in oxidative stress and BP. MMF was not found to alter blood Pb levels of animals. Red grape seed extract and ascorbic acid supplementation were also able to protect rats from Pb-induced (100 ppm) increased BP and heart rate, perhaps through the antioxidant properties of both the extract ([Badavi et al., 2008](#)) and vitamin C ([Mohammad et al., 2010](#)). Red grape seed extract did not alter the accumulation of Pb in blood, indicating that its protective effect was not mediated through altered Pb toxicokinetics; however, internal doses of Pb were not measured in the vitamin C study to clarify the mechanism of action of vitamin C. Another study found that the antioxidant, anti-inflammatory chemical, curcumin, as well as physical exercise training reversed Pb-induced increases in serum creatinine kinase-MB (CK-MB), low density lipoprotein (LDL), heart high-sensitivity C-reactive protein (hs-CRP), and MDA. Pb-induced decreases in serum total antioxidant capacity, high density lipoprotein (HDL), and heart glutathione peroxidase (GPx) were also reversed by curcumin and exercise. However, internal doses of Pb were not measured to clarify the mechanism of action in this study ([Roshan et al., 2011](#)).

Exposure to Pb can also affect the activity and levels of antioxidant enzymes. Male (♂) and female (♀) rats exposed to Pb for 18 weeks (100-1,000 ppm) had altered responses in antioxidant enzymes in heart tissue ([Sobekova et al., 2009](#); [Alghazal et al., 2008a](#)). Pb exposure in female rats increased the activity of cardiac SOD, GST, GR, and GPx (>100 ppm) and increased cardiac thiobarbituric acid reactive substances (TBARS, a measure of lipid peroxidation) (1,000 ppm). Pb exposure in male rats did not affect the activity of SOD or production of TBARS, however decreased the activity of GST and GR (>100 ppm). Male and female rats also accumulated different amounts of Pb in the cardiac tissue after similar Pb exposure (♂ 100 ppm: 205% of control, 1,000 ppm: 379%; ♀ 100 ppm: 246%, 1,000 ppm: 775%), which could explain the sex differences observed in antioxidant enzyme responses.

Oxidative stress can trigger a cascade of events that promote cellular stress, renal inflammation, and hypertension. As was shown previously ([Rodriguez-Iturbe et al., 2005](#)), Pb exposure can increase renal NF-κB, which was associated with tubulointerstitial damage and infiltration of lymphocytes and macrophages ([Bravo et al., 2007](#)). These events could also be ablated by MMF treatment, likely due to its anti-inflammatory and antioxidant properties. Pb also was found to induce inflammation in human endothelial cells as a model for vessel intima hyperplasia ([Zeller et al., 2010](#)). The pro-inflammatory cytokine, interleukin (IL)-8 protein and mRNA were increased, concentration- and time-dependently, after in vitro Pb exposure (5-50 μM). Enhanced IL-8 production was mediated through activation of the transcription factor Nrf2 (but not NF-κB, hypoxia inducible factor-1, or aryl hydrocarbon receptor), as shown through increased nuclear translocation and Nrf2 cellular knockdown experiments. Additionally, measures of endothelial stress, NQO1 and HO-1 protein, were induced by Pb exposure ([Zeller et al., 2010](#)). Pb treatment (20 ppm, i.p., 3 days/week, 8 weeks) increased the inflammatory markers hs-CRP and CK-MB in rat hearts ([Roshan et al., 2011](#)).

Oxidative stress affects vascular reactivity and tone through inactivation and sequestration of NO, causing a reduction in biologically active NO. Recent studies affirmed past conclusions on the interplay of ROS and NO metabolism in the cardiovascular effects of Pb. Elevated systolic BP and altered vasorelaxation after Pb exposure was accompanied by a decrease in total nitrates and nitrites (NO_x) ([Mohammad et al., 2010](#); [Zhang et al., 2007a](#); [Heydari et al., 2006](#)). Serum NO_x levels in Pb-treated rats remained depressed for 8 weeks and then reversed after 12 weeks, despite continued elevation in systolic BP ([Heydari et al., 2006](#)). This return of serum NO_x levels to levels similar in controls could be a result of compensatory increases in endothelial NOS (eNOS) attempting to replenish an over-sequestered NO supply. With this in mind, studies showed increased eNOS protein expression after long-term Pb exposure in kidney ([Zhang et al., 2007a](#)) and isolated cultured aorta ([Vargas-Robles et al., 2007](#)). No change

in inducible NOS was observed in isolated cultured aorta after 1 ppm Pb exposure ([Zhang et al., 2007a](#)). In contrast to long-term exposure, Pb treatment over a short time period (daily injections resulting in mean [SEM] blood Pb levels of 9.98 [1.7] µg/dL) was found to increase iNOS and phosphorylated eNOS protein ([Fiorim et al., 2011](#)) which may cause an increase in NO production and a short-term increase in NO bioavailability. This increase in NO bioavailability early after Pb exposure could be the immediate compensatory mechanism against the elevation in BP.

NO, also known as endothelium-derived relaxing factor, is a potent endogenous vasodilator. Toxicological studies continued to investigate the effects of Pb on NO-dependent vascular reactivity by using NO stimulating vasodilators, such as acetylcholine (ACh) and sodium nitroprusside (SNP), and NO inhibiting vasoconstrictors, such as L-NAME. Studies provided mixed evidence; however, results suggested that Pb disrupts the vasorelaxant response to NO in the aorta due to damage to the endothelium. Pb exposure (1 ppm and 100 µM, 1 hour) decreased ACh-induced vasorelaxation, which triggers the release of NO from the endothelial cell, in isolated rat tail artery, suggesting damage to the endothelium ([Silveira et al., 2010](#); [Zhang et al., 2007a](#)). In aortic rings of perinatally exposed rats (1,000 ppm through pregnancy and lactation, mean blood Pb level: 58.7 µg/dL), blocking NOS with L-NAME abolished the relaxant response evoked by ACh ([Grizzo and Cordellini, 2008](#)). However, there was no change observed in the relaxation response to ACh by Pb alone ([Fiorim et al., 2011](#); [Rizzi et al., 2009](#); [Grizzo and Cordellini, 2008](#)). Conversely, Skoczynska and Stojek ([2005](#)) found that Pb exposure (50 ppm; blood Pb level 11.2 µg/dL) enhanced NO-mediated vasodilation by ACh in rat mesenteric arteries, and NOS inhibition enhanced the ACh relaxant response. A number of studies found that Pb exposure did not affect smooth muscle integrity since SNP-induced vasorelaxation, which is endothelium independent, was unchanged ([Fiorim et al., 2011](#); [Silveira et al., 2010](#); [Rizzi et al., 2009](#); [Grizzo and Cordellini, 2008](#)).

NO also was found to play a role in the interaction between Pb and the vasoconstrictor response. Blocking NOS with L-NAME or inhibiting iNOS specifically, which decreases NO production, increased the contraction of aortic rings in response to the vasoconstrictor phenylephrine (PHE), and Pb exposure potentiated this response ([Fiorim et al., 2011](#)). Also, L-NAME increased the Pb pressor response to PHE after perinatal Pb exposure (1,000 ppm through pregnancy and lactation, blood Pb level 58.7 µg/dL) ([Grizzo and Cordellini, 2008](#)). Conversely, in rat renal interlobar arteries, Pb exposure blunted the increase in renal angiotensin II (AngII)-mediated contraction from NOS inhibition by L-NAME ([Vargas-Robles et al., 2007](#)). Treatment with the SOD mimetic tempol, which would increase NO bioavailability, decreased, but did not eliminate, the Pb pressor response ([Silveira et al., 2010](#)).

In summary, recent studies continued to provide evidence for the role of ROS in Pb-induced hypertension and vascular disease by indicating Pb-induced increases in ROS and modulation of cardiovascular responses by antioxidant substances. Additionally, recent studies continued to show that Pb-induced hypertension and vascular responses are mediated primarily via inactivation of NO not via inhibition of NO production.

Vascular Reactivity

Pb exposure-induced alterations of the adrenergic system, which can increase peripheral vascular resistance, and thereby arterial pressure, may be one mediator of Pb-induced hypertension. Pb exposure in animals can increase stimulation of the sympathetic nervous system (SNS), as shown by increased plasma levels of norepinephrine (NE) and other catecholamines ([Carmignani et al., 2000](#); [Chang et al., 1997](#)) and decreased β adrenergic receptor density and β agonist-stimulated cAMP production in the aorta and heart ([Tsao et al., 2000](#); [Chang et al., 1997](#)). These stimulatory effects on the SNS paralleled the effects of Pb on BP, cardiac contractility, and carotid blood flow. Pb-induced elevations in arterial pressure and heart rate were abrogated by ganglionic blockade ([Simões et al., 2011](#); [Lai et al., 2002](#)). Arterial pressure and heart rate gradually decreased 7 months after Pb exposure cessation as did the Pb-induced SNS alterations ([Chang et al., 2005](#)).

Increases in BP can be caused by activation of the SNS, which can lead to vascular narrowing, in turn, resulting in increased total peripheral resistance. In this neural mechanism, activation of the SNS leads to vasoconstriction, whereas inhibition leads to vasodilation. It has been suggested that Pb leads to increased vascular reactivity to catecholamines (i.e., epinephrine, NE, and dopamine), hormones of the SNS. Indeed, the isolated mesenteric vessel bed from Pb-treated rats (50 ppm with blood Pb level: 11.2 $\mu\text{g/dL}$, but not 100 ppm with blood Pb level: 17.3 $\mu\text{g/dL}$) exhibited increased reactivity to NE ([Skoczynska and Stojek, 2005](#)). However, in another study, 100 ppm Pb did not affect the NE-induced contractile response after 10 months of exposure ([Zhang et al., 2009a](#)), suggesting a small range of Pb doses affects pressor response to NE. Catecholamines act primarily through the adrenergic and dopaminergic receptors. Antagonists of $\alpha 1$ -adrenergic, $\alpha 2$ -adrenergic, β -adrenergic, and dopamine D1 receptors were found to abolish Pb-induced aortic contraction ([Fazli-Tabaei et al., 2006](#); [Heydari et al., 2006](#)). However, the $\alpha 1$ -adrenergic receptor agonist, PHE, induced aortic contractions and these were enhanced by treatment with Pb (100 ppm; blood Pb level: 26.8 $\mu\text{g/dL}$), indicating a specific role for the $\alpha 1$ -adrenergic receptor ([Silveira et al., 2010](#); [Grizzo and Cordellini, 2008](#); [Heydari et al., 2006](#)). Removal of the endothelium blunted the PHE-induced contraction. Conversely, short-term Pb treatment (7 days, i.p.) decreased the contractile response induced by PHE in rat aortas resulting in a decreased vascular reactivity ([Fiorim et al., 2011](#)). This decrease may represent a compensatory response

attempting to correct the Pb-induced BP elevation. Additionally, Pb blunted the isoproterenol-induced relaxation, supporting a role for the β -adrenoceptors in mediating the effects of Pb on vascular reactivity ([Vassallo et al., 2008](#); [Heydari et al., 2006](#)).

Recently, there was mixed evidence for Pb disrupting vascular reactivity to other pressor agents. Pb (1 ppm) treatment of isolated rat thoracic aorta increased 5-HT induced contraction, which was endothelium dependent, but not due to 5-HT_{2B} receptor expression ([Zhang et al., 2005](#)). Follow-up of this study in whole animals found, on the contrary, that Pb (100 ppm; blood Pb level: 28.4 $\mu\text{g/dL}$) decreased the maximum contractile response to 5-HT, but did not affect 5-HT plasma levels or 5-HT_{2B} receptor expression ([Zhang et al., 2009a](#)). In addition, Pb exposure (100 ppm, 12 weeks) increased the renal vascular response to AngII in isolated perfused kidneys from Pb-exposed rats ([Vargas-Robles et al., 2007](#)).

Studies continued to investigate the effects of Pb on NO-dependent vascular reactivity by using NO stimulating vasodilators, such as ACh and SNP, and NO inhibiting vasoconstrictors, such as L-NAME. These studies were discussed in a preceding subsection (Oxidative Stress Response).

Renin-Angiotensin-Aldosterone and Kininergic Systems

The adrenergic system also affects the renin-angiotensin-aldosterone system (RAAS), which is responsible for fluid homeostasis and BP regulation, and has been shown to be affected by Pb exposure. A meta-analysis found that Pb exposure (resulting in blood Pb levels: 30-40 $\mu\text{g/dL}$) increased plasma renin activity and renal tissue renin in young but not old rats ([Vander, 1988](#)). Exposure of experimental animals to Pb also induced increases in plasma, aorta, heart, and kidney angiotensin converting enzyme (ACE) activity; plasma kininase II, kininase I, and kallikrein activities; and renal AngII positive cells ([Rodriguez-Iturbe et al., 2005](#); [Sharifi et al., 2004](#); [Carmignani et al., 1999](#)). ACE activity declined over time while arterial pressure stayed elevated, suggesting that the RAAS may be involved in the induction, but not the maintenance of Pb-induced hypertension in rats.

Recent studies continued to implicate the RAAS in the development of Pb-induced hypertension, especially during early exposure in young animals. AngII, a main player in the RAAS, induces arteriolar vasoconstriction leading to increased BP. Pb exposure increased the vascular reactivity to AngII ([Vargas-Robles et al., 2007](#)). Acute (60 minutes) or short-term (7 days) treatment of rats to Pb increased the plasma ACE activity ([Fiorim et al., 2011](#); [Simões et al., 2011](#)), and Fiorim et al. (2011) additionally found this increase to be correlated with the Pb-induced increase in systolic BP.