

2.0 HAZARD IDENTIFICATION

Chapter 2 of the §403 risk analysis report presented information on the toxicity of lead, through a discussion of how body-lead burden is measured, how lead works in the body, the resulting adverse health effects, and populations at risk. This chapter introduced the endpoints used in the risk analysis to represent the adverse health effects resulting from lead exposure and to estimate the benefits of the §403 rule. These endpoints included the likelihood of exceeding a specified blood-lead concentration threshold (10 or 20 µg/dL), the likelihood of achieving a specified IQ score decrement as a result of lead exposure (1, 2, or 3 points on average across the population), the likelihood of achieving an IQ score in the population of less than 70 points due to lead exposure, and the average IQ score decrement in the population that results from lead exposure. The blood-lead concentration thresholds were among those established by the Centers for Disease Control and Prevention (CDC) as levels of concern. The IQ-related endpoints represented measures of the neurological effects of lead exposure. The representative population upon which the §403 risk analysis focused was children aged 1-2 years, as it was considered the most appropriate age range for the estimation of health effects.

In this chapter of the supplemental report, the results of four additional investigations into hazard identification are presented:

- Section 2.1: A review of the adverse health effects of lead exposure, with a focus on neurological effects, as observed in animal studies.
- Section 2.2: Support for the causality of adverse health effects due to lead exposure.
- Section 2.3: Characterizing the relationship between blood-lead concentration and IQ score.
- Section 2.4: Documenting what is known about the role that dust particle size and the chemical composition of lead compounds in lead-contaminated dust may play in determining the extent to which lead in residential dust is bioavailable to humans.

The motivation for including each of these sections into this chapter is presented within the introduction to each section.

2.1 REVIEW OF THE ADVERSE HEALTH EFFECTS OF LEAD EXPOSURE, WITH A FOCUS ON NEUROLOGICAL EFFECTS, AS OBSERVED IN ANIMAL STUDIES

The §403 risk analysis used data from human exposure studies to characterize the relationship between environmental-lead levels and measures of the various blood-lead concentration and health effect endpoints. However, as the SAB's review of the §403 risk analysis points out (USEPA,

1998b), causality is difficult to establish using human studies alone, due to the potential for confounding factors being present. For example, while average IQ score may differ significantly between one group of children with low blood-lead concentration and another group with elevated blood-lead concentration, the reason for this difference may not be solely due to lead exposure, but to demographic and other factors that cannot be controlled completely by the researcher. The factors left uncontrolled in the analyses of data from human exposure studies contribute to large uncertainties associated with these analyses. While ethical considerations preclude the use of humans in controlled lead-exposure experiments, a substantial amount of published literature is available on controlled lead-exposure experiments involving animals. Such studies use animals that are chosen from a homogeneous population, reared under identical conditions and randomly assigned to groups where the mode, duration, and amount of lead exposure is controlled within each group (these groups can include one or more control groups). Therefore, in a well-controlled animal study, the presence of significant differences between dose groups can be inferred to be the result of lead exposure at certain doses.

To address the SAB's recommendation to consider the findings of "... animal data, since they support human data by establishing causality, due to the absence of confounding variables, and potential mechanisms for adverse health effects" (USEPA, 1998b), this section presents the key findings of animal studies that have investigated the impact of lead exposure on adverse health effects, especially at low doses. In these studies, animals were typically exposed to lead either *in utero*, during infancy (through mother's milk/formula), during maturation or adulthood, or a combination of these life phases. The major lead-induced adverse health effects noted in humans, including neurological, neurodevelopmental, immunological, and systemic (e.g., cardiovascular, hematological, and renal effects) have been demonstrated in controlled, dose-response studies in rodents, dogs, and/or non-human primates. While this section recognizes the variety of adverse health effects, its primary focus is on the neurological effects of lead, as the §403 risk analysis has recognized that young children are most susceptible to neurological effects due to their developing central nervous systems.

A glossary of selected terms used in this section can be found in Appendix A.

2.1.1 Approach to Reporting the Findings of Animal Studies

This subsection reviews the incidence of adverse health effects associated with lead exposure, as reported in published animal studies. The emphasis of this review is on the neurological, developmental, and neurobehavioral effects of lead.

In preparing this review, it was not desired to duplicate the previous efforts of others who have prepared excellent published literature reviews that have been peer reviewed and are easily available to the general public. These include two articles cited by the SAB (USEPA, 1998b) as "important references" on animal studies data: Rice (1996) and Cory-Slechta et al. (1997). Other important review documents include USEPA (1986) and USDHHS (1999). This section frequently references the content of these and other study review documents.

To help identify any additional articles that may have been published since these key review references were prepared and published, a search of the scientific literature over the last five years was conducted. The strategy for this literature search was on the key words “lead,” “effect,” “exposure,” “neurologic,” “behavior,” “development,” “teratology,” and “animal” as whole or root words. Upon review of abstracts identified from this literature search, articles found to be relevant to the objectives of this report were obtained and reviewed, with high priority placed on study reviews.

Certain results of recent key studies identified within the review publications and the literature search are also discussed in detail in this section. Overviews of these studies are presented in Table 2-1. Note that these selected studies represent only a subset of all studies whose results and conclusions provide important contributions to the knowledge base on adverse health effects associated with lead exposure.

Section 2.1.2 focuses on the findings of animal studies on the neurological, developmental, and neurobehavioral effects of lead. This subsection contains an overview of the physiological consequences of these effects, along with a review of general findings of studies investigating these types of health effects. In addition, certain animal studies are discussed in greater detail with summaries of their design and conclusions.

Lead has been documented to have considerably more effects on the health of humans and animals than just neurological effects. Therefore, Section 2.1.3 presents a brief overview of general health effect information and how it relates to lead exposure, as observed in animal studies. Information in this subsection is organized according to the type of health effect.

2.1.2 Neurological, Behavioral, and Developmental Health Effects

Lead has been observed to have widespread neurotoxic effects, as well as to cause behavioral and cognitive symptoms, in humans. These effects are largely consistent with results of morphological, electrophysiological, biochemical, and behavioral studies on animals. Although lead toxicity research has isolated many of the specific neurological effects of lead, it is generally considered to be a relatively indiscriminate toxin within the neurological system. This consideration is largely due to the ability of lead to disturb several fundamental biotic processes such as cellular metabolism and energy production, ion transport across membranes, and protein function. In addition, the neurological effects of lead frequently have been observed to occur as a series of interrelated events. Thus, lead poisoning is likely to cause simultaneous and interrelated disturbances in a number of processes within the nervous system. As an additional consequence, the ability to separate the direct and indirect effects of lead on the neurological system is often difficult (Banks et al. 1997).

Table 2-1. Summary of Lead Exposure Levels and Key Findings for Selected Animal Studies

Authors	Subject Species	Lead Exposure	Key Findings / Effects of Lead Exposure
Altmann et al. (1993)	Rat	0 or 750 ppm lead acetate in diet at various life stages (<i>in utero</i> , pre-weaning, and post-weaning)	Active avoidance learning and long-term hippocampal potentiation were impaired when exposure occurred prior to 16 days postnatally.
Burger et al. (1998)	Turtle	0.25, 1.0, or 2.5 mg/g lead acetate in deionized water, by injection; one-time exposure; controls received an isotonic saline solution injection	Death with high dose; dose-dependent righting response impairment with low and moderate doses.
Bushnell et al. (1977)	Monkey	control, lower-dose (targeted blood-lead of 55 $\mu\text{g}/\text{dL}$), and higher-dose (targeted blood-lead of 85 $\mu\text{g}/\text{dL}$) of lead acetate in milk formula in the first year of life	At age 18 months, visual discrimination in the higher-dose group was impaired under dim light compared both to their performance under bright light and to the other groups under various luminescence levels.
Bushnell and Bowman (1979a, b)	Monkey	control, lower-dose (targeted blood-lead of 50 $\mu\text{g}/\text{dL}$), and higher-dose (targeted blood-lead of 80 $\mu\text{g}/\text{dL}$) of lead acetate in milk formula and food in the first year of life	In the first year of life, suppressed play and increased social clinging, as well as various social delays that occur when play environment is abruptly changed (primarily occurring in animals dosed post-natally only; 1979b). At age 4 years, when a subset of the animals was further tested, diminished performance on spatial-cue reversal learning sets was observed in the higher-dose group and, to a lesser extent, in the lower-dose group (1979a).
Chen et al. (1998)	Rat	0.2% lead acetate as drinking water; exposed <i>in utero</i> and during nursing via dams, and postweaning directly; controls received 0.145% sodium acetate in drinking water	Altered protein kinase C (PKC) distribution in the hippocampus.
Cory-Slechta (1997) (review)	Rat	50 or 250 ppm lead acetate as drinking water; exposed postweaning; control groups received no lead exposure	Disruption of neurotransmitter systems (i.e., dopamine and glutamine systems); selective learning deficits (e.g., impaired repeat acquisition performance); dose-dependent alteration of fixed interval schedule-controlled response rates.

Table 2-1. (cont.)

Authors	Subject Species	Lead Exposure	Key Findings / Effects of Lead Exposure
Cutler (1977)	Rat	0 or 0.1% lead acetate exposed pre-weaning, and 0 or 0.1% lead acetate exposed post-weaning in drinking water	At 8 weeks of age, duration of non-social activity was significantly greater in the exposure group versus the controls for males but not females. Across both sexes, frequency and duration of social and sexual investigation was decreased in the exposure group.
Fox et al. (1997)	Rat	0, 0.02%, or 0.20% lead acetate in diet at various life stages (<i>in utero</i> , pre-weaning, and post-weaning)	Significant association between retinal degeneration and both age and lead exposure level.
Hastings et al. (1977)	Rat	0, 0.02%, or 0.10% lead acetate in diet pre-weaning	At 60 days of age, aggressive behavior was significantly reduced in the exposed groups, but no significant differences were observed in visual discrimination tasks.
Kuhlman et al. (1997)	Rat	750 or 1000 ppm lead acetate, as feed; exposed <i>in utero</i> , <i>in utero</i> to adulthood, or postweaning to adulthood; controls received no lead exposure	Performance impairment in water maze for all rats exposed <i>in utero</i> ; no impairment for rats exposed post-weaning.
Lasky et al. (1995)	Monkey	Controls or "modest" exposure to lead via lead acetate <i>in utero</i> , pre-weaning, and/or post-weaning within the first year of life	Markedly abnormal distortion product otoacoustic emissions (DPEs) for the two animals with the highest blood-lead concentrations. Otherwise, DPEs did not differ significantly between the groups, although auditory brain stem evoked response (ABR) did differ significantly.
Lilienthal et al. (1990, 1994)	Monkey	0, 350, or 600 ppm lead acetate in diet of mothers and their offspring (<i>in utero</i> , pre-weaning, and post-weaning to age 10 years)	At age 7 years, offspring in the exposed groups had significantly higher latencies in flash-evoked and brain stem auditory evoked potentials (BAEP) which increased with increasing click rates (1990). At age 12 years, offspring in the exposed groups had significantly increased amplitudes of the scotopic b-wave in electroretinogram (ERG) recordings (1994).
Mello et al. (1998)	Rat	1.0 mM lead acetate as drinking water; exposed <i>in utero</i> and during nursing via dams; control dams received deionized water only	Selective motor skill impairment (accelerated fist eye opening, startle reflex, and free-fall righting; impaired spontaneous alternation performance in maze).

Table 2-1. (cont.)

Authors	Subject Species	Lead Exposure	Key Findings / Effects of Lead Exposure
Nagymajtenyi et al. (1998)	Rat	80, 160, 320 mg/kg/day lead acetate in distilled water, by gavage; exposed pre- or post-natally; controls received same volume of distilled water by gavage	Dose dependent increase in behavioral and bioelectric aberrations (e.g., hyperactivity).
Rice and Gilbert (1990a, 1990b)	Monkey	1.5 mg/kg/day lead acetate, in capsules; exposed continuously after birth, postnatally until 400 days, or after 300 days from birth; controls received vehicle only	<p>Nonspatial discrimination tests (1990a): group dosed continuously from birth onward exhibited greatest degree of impairment, followed by group dosed after infancy only (no impairment observed in group dosed during infancy only).</p> <p>Delayed alternation task (1990b): all exposed groups showed impairment to an approximately equal degree.</p>

Research, based on both human observation and animal studies, indicates that relatively low doses of lead can adversely affect both the peripheral and central nervous systems, while high lead exposures can result in acute lead encephalopathy, and may ultimately lead to death. Lead induced damage to the brain and nervous system may be manifested as various and diverse developmental symptoms, including both behavioral and cognitive impairments.

In the following subsections, the physiological effects of lead on the brain and nervous system (Section 2.1.2.1) and subsequent effects on development and behavior (Section 2.1.2.2) are discussed. The findings of specific studies listed in Table 2-1 above are included in this discussion. In general, some caution must be taken when extrapolating the findings in these studies to humans and to other species. For example, lead neuropathy in rats is primarily characterized by demyelination of nerves, while cats, rabbits, and humans generally show damage to the axons of nerves (Davis et al., 1990). Developmental age of the brain also varies between animal species and humans. For example, at birth, the rat brain is relatively less developed and is roughly equivalent to the human brain at 5-6 months of gestation (Winneke et al., 1996). Furthermore, although rats and possibly monkeys tend to have higher tolerances for the general toxic effects of lead exposure relative to humans (i.e., they require a higher exposure level to reach an equivalent blood toxicity level), the lowest blood-lead levels at which lead-induced developmental/neurobehavioral effects have been observed in animals and in humans are reported to be similar in magnitude (Banks et al., 1997; Davis et al., 1990; USDHHS, 1999).

2.1.2.1 Physiological Effects of Lead on the Neurological System.

Overview. As lead is known to adversely affect several universal processes within biological systems, the symptoms of lead poisoning have been observed in many cell types, tissues, and organs within the neurological system.

On the cellular level, lead has been observed to cause disruption of mitochondrial function (i.e., cellular metabolism), damage to cell structural components (e.g., microtubules), and damage to glial cells and the myelin sheaths and axons of nerve cells (USEPA, 1986). Electrophysiological and biochemical processes at the cellular level may also be disrupted by lead exposure. Specific alterations of these processes reported in animal studies include impairment of synaptic events and neuron function, interference with neurotransmitter function, and protein activity inhibition (e.g., enzymes and hormones) (USEPA, 1986). Much of lead's role in cellular level dysfunctions is suggested by many researchers to be attributed to its interference with calcium-mediated processes (Banks et al., 1997). Calcium is an important ion in many biological systems and is specifically involved in neurological phenomena such as enzyme-protein activation, secondary messenger regulation of metabolic pathways, membrane potential/ion channel regulation, and neurotransmitter release. Lead ions, as they are similar to calcium ions in both size and charge, can substitute for calcium and thus competitively interfere with these types of calcium-mediated cell processes.

Disruption of cellular processes can eventually result in damage to tissues and organ systems within the neurological system. Reported effects of lead in animals at the organ and system level within the central nervous system include compromise of the blood-brain barrier, disruption of the limbic system and cerebral cortex, and damage to the cerebellum (Banks et al., 1997; USEPA, 1986). Animals exposed to lead in early post-natal life have also exhibited reductions and delays in development of various brain regions, including the hippocampus and cerebral cortex (Banks et al., 1997; USDHHS, 1999). The observed effects of lead exposure on specific organ systems and processes, as observed in animal studies, are now discussed.

Blood-brain barrier. Banks et al. (1997) reviewed animal studies that examined the effect of lead exposures on the blood-brain barrier that regulates the movement of chemical substances in and out of the brain. In studies of rats exposed to relatively high lead levels, higher concentrations of lead were observed in the barrier capillaries than were observed in the brain as a whole. USEPA (1986) reviewed studies which provided evidence that lead transport within the brain is by the same mechanisms as calcium transport. Thus, as the capacity for calcium transport (specifically, into neural cell mitochondria) is known to be much higher in the brain than other body tissues, a lead accumulation in brain capillaries is not unexpected (USEPA, 1986). Acute lead toxicity as lead accumulations in brain capillaries may disrupt barrier permeability, allow greater influxes of water, ions, and other substances, and result in swelling of the brain (encephalitis) (USEPA, 1986). Although lower lead exposure levels (< 40µg/dL) have not been reported to result in specific damage to the barrier or in disproportionate accumulation of lead in the capillaries of the blood-brain barrier (Banks et al., 1997),

developing brains have been suggested to be particularly susceptible to the transfer of even very low levels of lead from the blood into the brain since the blood-brain barrier is not yet fully functional (Altmann et al., 1993; Cory-Slechta, 1997).

General cellular processes. Several *in vitro* studies with animal cells have provided evidence that a major site of lead interference is with cellular metabolism and energy transfer via disruption of the normal mitochondrial ion gradient (Banks et al., 1997; USEPA, 1986). In addition, mitochondria in the cerebellum and in developing brains of animals have been observed to show a greater sensitivity to lead disruption than mitochondria in other body tissues or at other ages (USEPA, 1986). This has been suggested to provide a possible explanation for one of the root causes of the greater sensitivity of the neurological system, and of the young in particular, to lead poisoning (USEPA, 1986). Impairment of mitochondrial energy production subsequently can affect many other energy-requiring cellular processes, such as protein synthesis, lipid synthesis, and membrane integrity. In animal studies, the normal development of proteins in neurons was altered in rats exposed to lead perinatally (USDHHS, 1999). Other studies reviewed by Banks et al. (1997) indicate that moderate levels of lead can interfere with microtubule formation (*in vitro* animal cell studies) and the formation of the myelin sheath in neurons in both the central and peripheral nervous systems of lead exposed rodents.

Lead can also interfere directly with calcium-mediated cell processes. This may include disruption of protein function (e.g., enzyme regulation of cell growth and differentiation), ion transport systems across membranes, and membrane potentials (Banks et al, 1997; USEPA, 1986). For example, a study performed by Chen et al. (1998) found that hippocampal protein kinase C (PKC) activity, which has been correlated with performance in several learning tasks, was altered by relatively low internal lead exposures (<30 µg/dL) in postnatal rats. More information on this study and its findings can be found in the discussion below on the limbic system and hippocampus.

Neuron and neurotransmitter function. Lead-induced disruption of cellular processes may result in neuron dysfunction. According to a body of experimental animal research reviewed by Banks et al. (1997), evidence exists that moderate to low levels of lead exposure can impair synapse formation in the hippocampus during postnatal development of rats, in the visual cortex of primates, and in the frontoparietal cortex of guinea pigs, and also appears to interfere with synaptic transmission in fetal rat hippocampal neurons by blocking postsynaptic receptors. USEPA (1986) reviewed numerous studies in which rats exhibited morphological effects such as decreased glial cell and synaptic density, and delayed maturity of synapses in neurons of the cerebral cortex with lead exposure. Several of the reviewed studies also reported abnormal development of neuron dendrites and persistent impairment of electrical activity (i.e., reduced firing rates) in the cerebellum of cats and rats exposed perinatally to low levels of lead (Banks et al., 1997). The cerebellum is responsible for the regulation and coordination of complex voluntary muscular movement, and is also implicated in cognitive attention-switching activity. The cerebral cortex is largely responsible for higher brain functions, including sensation, voluntary muscle movement, thought, reasoning, and memory. Thus, damage to neurons in these areas may

explain some attention deficits, learning and memory impairments, and disturbances in motor coordination that have been observed in behavioral studies of lead exposure (Banks et al., 1997).

Lead has also been observed to interfere with the release and uptake of neurotransmitters in various *in vitro* and *in vivo* animal studies, including the inhibition of neurotransmitter release from calcium sensitive voltage channels in snails, and alteration of synthesis and turnover rates of various neurotransmitters in different regions of the brain (hippocampus, cerebellum, hypothalamus, brainstem) in lead-exposed rats (Banks et al., 1997; USEPA, 1986; Nagymajtenyi et al., 1998). Altered activity of various neurotransmitters has been observed in studies involving rats exposed to lead prenatally and postnatally (USDHHS, 1999). In the reviewed literature, lead has generally been reported to have highly variable and non-specific effects on the various neurotransmitter systems, possibly due to its more general effect on metabolic processes (Banks et al., 1997).

Cory-Slechta (1997) conducted a review of studies linking disruptions in neurotransmitter systems (i.e., dopaminergic (DA) and glutamatergic (GLU) systems) with behavioral and cognitive impairments in lead exposed animals. The author cites previous research which indicates that the DA and GLU neurotransmitter systems are critical to various cognitive functions, and also are sensitive to lead-induced disruptions. In addition, the author reviews studies reporting non-lead related disruptions of these systems and the concurrent appearance of behavioral symptoms that are very similar to symptoms of lead toxicity. However, the author also concedes that much of the historical research linking behavioral impairments to biochemical effects is based solely on correlations and that relationships are complicated by the fact that any given behavioral symptoms may have multiple physiological mechanisms. Cory-Slechta (1997) summarized several studies conducted in her own lab on rats exposed, postweaning (i.e., at 21 days of age), to lead (0, 50 and 250 ppm lead as lead acetate) in drinking water for varying durations of time. Results of these studies indicated that the DA neurotransmitter system is vulnerable to lead-induced modifications, such as impaired regulation of DA synthesis and release. These modifications to DA system function were suspected to contribute to alterations in response control (fixed interval schedule-controlled behavior) that were observed in lead-exposed rats in the reviewed studies. Results indicated that the GLU system was also involved in lead-induced impaired learning, primarily as manifested by an increase in perseverative errors in lead-exposed rats, relative to controls. There was no evidence that the DA system was involved in learning accuracy. The author suggests that confirmation of the involvement of the GLU and DA systems in lead-induced effects, could also have implications extending beyond cognitive concerns. For example, similar patterns of neurotransmitter disruptions have been associated with schizophrenia, drug addiction, and psychosis (Cory-Slechta, 1997).

Lead-induced disruption of neuron and neurotransmitter function can result in altered bioelectric activity in the brain and nervous system. This was seen, for example, in a study by Nagymajtenyi et al. (1998) in which 120 female and 60 male rats and their 120 male offspring were administered a lead acetate solution by gavage at a concentration of either 0, 80, 160, or 320 mg/kg. There were three variations on the treatment schedule: (1) pregnant females were dosed only during the

5th -15th day of pregnancy; (2) pregnant females were dosed during pregnancy and lactation; or (3) pregnant females were dosed during pregnancy and lactation, and their weaned offspring were dosed for 8 weeks. Behavioral observations of the offspring were made at 12 weeks. The study observed electrophysiological disruptions, including changes in electrocorticogram (ECoG) indices, cortical evoked potential, and slowed nerve conduction velocity, in the somatosensory area of the cerebral cortex in the rats exposed to lead both pre- and post-natally relative to controls. Electrophysiological functions showed both dose- and treatment-dependent changes, including decreased mean amplitude and increased frequency of the ECoG, and lengthened latency and duration of the evoked potentials. The observed changes in electrophysiological functioning depended on the dose and timing (i.e., age of animal at exposure) of lead administration. The authors suggested that non-invasive monitoring of electrical disturbances in the nervous system may provide a valuable and early indicator of low-level lead poisoning.

The limbic system and hippocampus. The limbic system (e.g., hippocampus) is of particular interest in lead toxicity studies as it is key in many of the processes that appear to be affected by lead poisoning, including cognition, emotion, motivation, behavior, memory, and various autonomic functions. Some researchers have even suggested that symptomatic similarities (e.g., learning and memory impairments) between lead toxicity and other experimental limbic system disruption indicate that the limbic system is a target site for lead toxicity in the brain (Walsh and Tilson, 1984).

Reported behavioral changes that may be attributable to hippocampal damage include increased aggressiveness, seizures, inappropriate responsiveness, reversal problems, visual discrimination deficits, impaired motor coordination, and other types of learning deficiencies (Petit et al., 1983). Furthermore, the developing hippocampus may be more susceptible to functional injury by lead exposure compared to the mature hippocampus. For example, Altmann et al. (1993) conducted a study where 88 female rats were dosed with lead acetate in their diet at either 0 or 750 ppm for 50 days prior to mating through day 16 following birth of a litter, at which time 2-3 male offspring were taken from each litter and dosed under the same regimen until sacrifice. Half of the animals were tested for two-way active avoidance learning. This study observed a correlation between hippocampal disruptions and active avoidance learning deficit for rats exposed to lead during the prenatal and early postnatal stages (i.e., during hippocampal development), but not when lead exposure occurred only after 16 days postnatally.

Some studies reviewed in the literature differ on the extent to which the hippocampus is a target organ for lead. For example, relative to other regions of the brain, greater impairment of neuron function has been observed in hippocampal cells of rats exposed to lead perinatally (Banks et al., 1997). Studies reviewed by Petit et al. (1983) indicated that higher levels of lead may tend to accumulate in hippocampal cells of rat brains. However, other studies reviewed by Banks et al. (1997) did not report a preferential accumulation of lead in the hippocampus of young lead-exposed rats. For example, a 1994 study by D.V. Widzowski and D.A. Cory-Slechta (as cited in Banks et al., 1997) exposed rats postnatally to lead from dams' milk (dosed with four levels of lead from 100 to 2000 ppm

lead) and measured lead levels in 12 brain regions after 7-60 days. The study found that lead tended to accumulate in similar patterns across brain regions and exposure levels. Some researchers have hypothesized that the particular vulnerability of the hippocampus to lead poisoning may be due more to sensitivity rather than increased lead accumulation in this region (Banks et al., 1997). In addition, because the most rapid phase of development of hippocampus is known to occur postnatally (late compared to other brain regions), a particular sensitivity of the hippocampus to early-life lead exposure is plausible (Petit et al, 1983; Altmann et al., 1993).

Specific lead-associated physiological effects in the hippocampus, as reported in animal studies reviewed by Banks et al. (1997) and Petit et al. (1983), include significant reductions in size and weight of the hippocampus and reductions in hippocampal cell layer thickness with relatively high perinatal lead exposure in rats. Damage and stunting of hippocampal structural cells (glial cells/astrocytes) was observed in rats and monkeys exposed to lead prenatally and postnatally. Perinatally exposed rats also exhibited reductions in development of hippocampal neuronal dendrites and mossy fiber pathways, which are both involved in the transmission of nerve impulses (Petit et al., 1983).

Initially mentioned in the overview of general cellular processes above, Chen et al. (1998) performed a study to investigate the effects of developmental lead exposure on protein kinase C (PKC) activity in the hippocampus of rats at various postnatal ages. This study attempted to elucidate some of the physiological mechanisms of lead-induced learning deficits. In this study, lead was administered orally as 0.2% lead acetate in drinking water to pregnant and lactating female rats and then directly to their weanling pups (weaned at postnatal day 21) in drinking water. Controls received 0.145% sodium acetate in drinking water. Four to six rat pups were randomly selected for necropsy from different dams at postnatal days 7, 14, 28, and 56, and PKC activity was measured in both the membrane and cystolic fractions of the hippocampi. Results showed that lead exposure increased PKC activity in the cystolic fraction at postnatal day 56, and decreased PKC activity in the membrane fraction at postnatal day 7. The ratio of membrane to cystolic PKC activity, which is indicative of PKC distribution, decreased at postnatal days 28 and 56.

A review of studies in Chen et al. (1998) indicated that PKC activity has been associated with various brain functions (e.g., ion channel function, receptor function, and neurotransmitter release) and that alteration of hippocampal PKC, in particular, has been correlated with poor performance in several learning tasks. Therefore the authors hypothesize that the lead-induced alterations of PKC activity and distribution observed in their study may have caused functional changes in the animal brain, including modulation of ion channels, desensitization of receptors, and enhancement of neurotransmitter release. Chen et al. (1998) also suggested that some of the learning and memory deficits observed in children are likely to be causally related to the types of PKC activity alterations exhibited in this study.

The visual system. Some researchers contend that the retina serves as a good model for studying the effects of lead on the central nervous system. Because most retinal cells, like the central nervous system, develop during gestation and, in the rat, for up to two weeks postnatally, researchers

have studied the effects of lead exposure on retinal cell development in post-natal rats in order to characterize such effects for humans during early gestation and post-natal periods. Fox et al. (1997) studied the effects on retinal development of low and moderate lead exposure via mother's milk in female Long-Evans hooded rats aged 0 to 21 days (i.e., from parturition to weaning). These rats were partitioned into three dose groups (6-14 rats per group) based on the concentration of lead (0, 109, or 1090 ppm) in the lead acetate solution of drinking water that the dams were provided. At 21 days of age, lead levels in the blood and the retinas of rats in both the low and moderate dose groups averaged significantly higher than the control group, while significantly higher results were seen at 90 days of age in only the high dose level group and in only retina-lead levels. Blood-lead levels averaged 19 and 59 $\mu\text{g/dL}$ in the low and high dose groups at 21 days of age compared to 1 $\mu\text{g/dL}$ in the control group. Significant retinal degeneration (i.e., rod and bipolar apoptotic cell death) was associated with age and lead exposure levels in this study. The higher loss rate of rods in the lead-exposed groups was associated with a loss of rhodopsin content, implying that the loss was directly due to the presence of lead. The authors concluded from these results (when considered with the results of other researchers) that the developing retina may be more sensitive to lead exposure in pre-weaned rats than the hippocampus.

Also adopting the hypothesis that the effects of lead on the central nervous system leads to adverse effects on the eye, Bushnell et al. (1977) document the findings of experiments to characterize the relationship between high food-lead exposures early in life and impaired scotopic visual function (i.e., night blindness). In their experiments, baby formula was spiked with lead acetate and given daily to six rhesus monkeys in their first year of life. Lead consumption was regulated to allow blood-lead levels to be maintained at an average of 55 $\mu\text{g/dL}$ for three monkeys and 85 $\mu\text{g/dL}$ for three monkeys. Four other monkeys whose formula was not spiked with lead served as a control group. Approximately 18 months after this feeding paradigm was ended, monkeys in all three groups averaged nearly normal blood-lead levels. At this time, the monkeys were administered a discrimination procedure at various light levels to test their ability to select an option which provided reinforcement (i.e., food) versus an option which did not provide reinforcement. Animals exposed to the higher levels of lead in the first year of life (i.e., the 85 $\mu\text{g/dL}$ group) performed significantly worse in this procedure compared to animals in the control and lower lead groups as light levels were reduced. Various controlling factors within this experiment allowed the researchers to conclude that the primary reason for the degraded performance in the higher-dosed animals was most likely a loss of scotopic function. Thus, the researchers concluded that lead exposure early in life was associated with impaired scotopic visual performance later in life, even when blood-lead levels in these animals were allowed to return to normal levels.

In a study performed by Lilienthal et al. (1994), 15 rhesus monkeys were pre- and post-natally exposed to lead at one of three levels (0, 350, 600 mg/kg lead acetate) until they were nearly 10 years of age. At approximately 12 years of age, electroretinogram (ERG) recordings were made of each eye in these monkeys. Five animals were in the control group (average blood-lead concentration of 0.44 $\mu\text{g/dL}$), four in the lower-dosed group (average blood-lead concentration of 4.55 $\mu\text{g/dL}$), and six in the

higher-dosed group (average blood-lead concentration of 8.26 µg/dL). Significant differences (at the 0.05 level) were observed across dose groups in the amplitudes (but not latencies) of the scotopic b-wave, with increased amplitudes seen in the two lead-dosed groups, and the nature of the differences being dependent on luminescence level. The lead-induced effects were similar to those observed earlier in these animals and were similar to the effect of dopamine antagonists. This suggests that lead may be permanently affecting dopaminergic processes.

Over many years, significant visual impairment associated with lead exposure has been observed at high lead levels in rabbits, rats, and monkeys, both at the retina and visual cortex (Otto and Fox, 1993). Exposure to moderate levels of lead by rats can result in decreases in rod cells, thinning of retinal layers, reductions in the number of axons in the optic nerve, and necrosis of photoreceptors and cells in the inner retinal layer (Banks et al., 1997; USEPA, 1986). While such damage is less frequently associated with low levels of lead exposure, functional and neurochemical effects on the retinal system can be associated with low-level lead exposures in rats (i.e., blood-lead concentrations below 20 µg/dL; Otto and Fox, 1993), along with persistent decreases in visual acuity and spatial resolution (USDHHS, 1999). While considerable data exist to allow the neurotoxicity of lead to be characterized, considerably less data exist on the morphological effects of lead on the visual system, especially at low exposure levels. In addition, Otto and Fox (1993) have concluded that the effects of lead are more likely to adversely affect rod cells compared to cone cells.

Davis et al. (1990) reviewed several studies in which lead exposed rats and monkeys exhibited decreased responsiveness of neurons to visual stimuli, as assessed by parameters (i.e., visual evoked potentials) which measure nerve conduction. Lilienthal et al. (1990) found that central visual processing, as reflected in measurements of visual evoked potentials (VEP), was clearly affected in lead exposed monkeys. Monkeys were exposed to dietary lead pre- and postnatally (low lead group at 350 ppm and high lead group at 600 ppm per day), and VEP was measured in response to visual flash stimulation beginning at age seven. Results showed that amplitudes of sensory-evoked potentials were smaller and latencies were longer in lead-exposed monkeys relative to controls, even in monkeys with the lower lead exposure regime.

The study by Nagymajtenyi et al. (1998) introduced earlier in this subsection also observed disruptions in the functioning of optical nerves in prenatally and postnatally lead-exposed rats. In their study, effects of lead exposure were most often significant in the middle and high lead exposure groups, and included dose dependent changes in visual evoked potentials and slowed nerve conduction velocity, as measured in response to flash stimulation.

The auditory system. In a review of the literature (on both human and animal studies) on the effects of lead exposure on the auditory system, Otto and Fox (1993) concluded that lead exposure is more likely to adversely affect that portion of the auditory system that resides within the central nervous system (e.g., cochlear nerve) compared to more peripheral sites where sensory transduction processes occur. However, more data were deemed necessary to more definitively characterize the effects of

lead on specific sites within the auditory system and how such auditory dysfunction contributes to a child's overall learning impairment that can be attributed to lead exposure.

Certain animal studies have reported an association between lead exposure and disruptions of the auditory system. For example, Lilienthal et al. (1990) observed lead-induced alterations in auditory functioning in monkeys, as reflected in measurements of brainstem auditory evoked potentials (BAEP). In their study, monkeys were exposed to dietary lead pre- and postnatally (low lead group at 350 ppm and high lead group at 600 ppm per day), and BAEP was measured in response to auditory click stimulation beginning at age seven. Results showed that BAEP latencies increased with increasing click rates in lead-exposed monkeys relative to controls, although the increase tended to be consistent in the group of monkeys with the higher lead exposure regime (600ppm). The study by Nagymajtenyi et al. (1998) (cited earlier in this subsection) reported other types of disruptions in the auditory pathway in prenatally and postnatally exposed rats, including changes of auditory evoked potential and slowed nerve conduction velocity, as measured in response to click stimulation.

To assess the long-term auditory effects of chronic lead exposure at low levels, Lasky et al. (1995) assessed auditory functioning in two groups of 11-year-old rhesus monkeys, where one group contained 11 monkeys who were exposed to lead either pre- or post-natally (within the first year of life), and the other group contained 8 monkeys who were not exposed. Auditory function in these monkeys was assessed by measuring distortion product otoacoustic emissions (DPEs), auditory brain stem evoked responses (ABRs), and middle latency evoked responses (MLRs). The two animals with the highest blood-lead concentrations during their first four years had "markedly abnormal" DPEs, where this result was found not to be due to canal obstruction nor middle ear problems. Among the remaining animals, DPEs did not differ significantly between the two groups, although DPE amplitudes increased more rapidly (given the stimulus level) in the control group compared to the exposed group. The ABRs, but not the MLRs, differed significantly between the two groups of animals. The exposed animals tended to have slightly longer latency ABRs.

2.1.2.2 Behavioral and Developmental Effects of Lead. Several recent review papers have summarized previous research and advances in the area of neurotoxicological effects of lead. The findings of key studies and of study reviews by Banks et al. (1997), Cory-Slechta (1997), Davis et al. (1990), and Rice (1996), among others, are presented below. When possible, attention was focused on health effects at low levels of lead exposure or at low blood-lead levels. Many animal studies have investigated the neurobehavioral effects of lead exposure, that is, effects upon learning and performance activities. Some studies also addressed related issues such as: (1) what blood-lead levels were associated with learning and performance deficiencies in test animals; (2) whether other signs of toxicity were present; (3) what biochemical mechanisms produce toxicity; and (4) how blood levels in exposed animals correlate with specific blood-lead levels in humans at which analogous effects upon learning/IQ are observed. Many experiments utilize rats or monkeys as subjects since they respond to motivational factors, usually food rewards, and can be induced to learn certain behaviors of interest to researchers.

To synthesize the neurobehavioral effects of lead across species, Davis et al. (1990) conducted a review of rodent, primate, and human studies. The lowest levels of internal lead exposure during early development at which neurobehavioral effects have been observed were reported to be <20 µg/dL for rodents (Cory-Slechta et al., 1985) and 15-25 µg/dL for primates (Rice, 1985). These ranges are similar to the lowest range (10-15 µg/dL) in which adverse neurobehavioral effects have been observed in children (Davis et al., 1990; Fulton et al., 1987; Silva et al., 1988; USEPA, 1999).

Rice (1996) has summarized results from both human epidemiology and animal studies of the potential behavioral and developmental effects of lead exposure as follows: “Increased distractability, inability to inhibit inappropriate responses, perseveration, and inability to change response strategy are common themes that may be extracted from both literatures.” These observations are noted in the findings presented throughout this subsection.

Learning deficiencies and behavioral test performance. To investigate the association between lead exposure and learning deficiencies, Rice (1996) conducted an extensive review of animal studies, interpreted in conjunction with learning disabilities in children as reported in human epidemiological studies. In experiments with rats or monkeys, a general learning deficiency was frequently observed at high lead exposure levels. However, for monkeys exposed to low or moderate lead levels, the majority of the impairment was evident only with the performance of more complex tasks, such as non-spatial discrimination reversal tasks. This phenomenon was observed in (a) monkeys exposed to lead from birth with preweaning blood-lead levels of 50 µg/dL and adult blood-lead levels of 30 µg/dL (Rice, 1988); (b) monkeys with blood-lead levels of 30-35 µg/dL from infant formula and postweaning levels of 19-22 µg/dL (Rice and Gilbert, 1990a); and (c) monkeys with blood-lead levels of 15-25 µg/dL during infancy and steady-state levels of 11-13 µg/dL during adulthood (Rice, 1985). In these studies, lead exposure had ceased by the time performance tests were conducted.

Kuhlman et al. (1997) conducted an experiment with 10 rats reared in each of five groups. The control group received no lead exposure. The “maternal exposure” group was exposed to lead *in utero* and during lactation (750 ppm lead acetate in feed via dams), but was moved to a control diet after weaning. The permanent group was exposed to lead both *in utero* and continuously afterward into adulthood (750 ppm lead acetate in feed). Finally, there were two post-weaning groups, in which no exposure occurred until after weaning, and then pups were fed diets containing two different lead concentrations (750 or 1000 ppm lead acetate in feed). Rats were performance-tested using a water maze at about 100 days of age, which tested their ability to locate a submerged platform, and their blood-lead levels were measured at that time. Even though average blood levels in that group (1.8 µg/dL) had returned to control levels by the time of the test, a highly significant impairment in performance (e.g., longer time to find platform, longer pathway to platform) was observed in both the maternal and permanent exposure groups. The post-weaning exposure groups did not show any significant performance impairment, even though average blood-lead levels exceeded 20 µg/dL at the

time of testing. The authors suggested that the results seen in the maternal group demonstrated the impact of lead upon early development.

In a study documented by Mello et al. (1998), rat pups were exposed to lead *in utero* and during nursing, using 1.0 mM lead acetate administered to dams as drinking water. Eleven litters from control females and nine litters from lead-exposed females were used, for a total of 160 pups. The pups were observed for physical development and tested for reflexes/behavior at aged 17-19 days. While lead exposure appeared to significantly accelerate the appearance of first eye opening, startle reflex, and free-fall righting, it significantly impaired spontaneous alternation performance in a maze. No explanation was given for the seemingly contradictory effects in this study, though the authors suggested that any lead-induced alterations in animal development or behavior, regardless of direction, must be considered deleterious.

Operant discrimination and reversal tasks. Rice (1996) describes experiments that were conducted with visual discrimination problems with the addition of “reverse performance” requirements, and/or the addition of irrelevant distracting signals. In the operant discrimination reversal task, the researcher changes the pattern of rewards so that previously-learned correct and incorrect responses become switched. Lead-exposed animals sometimes perform as well as controls in the original learning acquisition, but perform poorly when the change of rules requires learning a change in strategy. Also, lead-exposed animals tend to be distracted by irrelevant details more than do controls (although in some cases they may perform similar to controls in the absence of such distraction). For example, Gilbert and Rice (1987) report an experiment in which monkeys exposed to low lead levels (50 and 100 $\mu\text{g}/\text{kg}/\text{day}$) through age 10 years tended to perform poorly, relative to controls, on spatial discrimination reversal tasks with unfamiliar distracting cues, but adequately on tasks with familiar distractions. Furthermore, the lower dose group was impaired only during the tasks immediately after the introduction of the irrelevant stimuli, but not after the irrelevant stimuli became familiar. Under these dosing regimes, steady-state blood lead concentrations in these mature adult monkeys (13.1 $\mu\text{g}/\text{dL}$ for the higher dose group and 10.9 $\mu\text{g}/\text{dL}$ for the lower group) approximate levels typical for humans in industrialized environments (Gilbert and Rice, 1987).

Bushnell and Bowman (1979a) reported diminished performance on spatial discrimination reversal tasks by adult monkeys (4 years of age) that were exposed to dietary lead in the first year of life (either 0.287 mg/kg or 0.880 mg/kg per day as lead acetate in formula). This finding was observed despite average blood-lead levels in each group being essentially normal at the time of testing.

Impairments in learning and performance have also been noted in experiments with lead-exposed rats. Cory-Slechta (1997) found that selective learning deficits were present after lead exposures of 50 and 250 ppm (as lead acetate in drinking water) with resulting blood-lead levels as low as 20-25 $\mu\text{g}/\text{dL}$. Lead-exposed rats performed as well as controls during the performance component of the experiment (i.e., the correct sequence of responses remained constant across trials), but less accurately during the repeat acquisition component (the correct sequence changed in an unpredictable

way with each new set of trials). Rats also displayed perseverative behavior, pressing the same lever repeatedly, even though the experiment precluded this pattern of response from generating a food reward. The author discusses the research attempting to elucidate some of the biochemical mechanisms underlying these results, including evidence which strongly suggests the existence of a link between learning impairments and lead-induced disruptions of neurotransmitters, particularly those of the glutamine system.

Cory-Slechta (1997) also reviewed the reported effects of lead on fixed interval (FI) test performance (delayed response operant schedule of food reinforcement). The FI test requires the animal to bar-press only at specific minimum time intervals before a food reward can be provided. Studies conducted by Cory-Slechta showed that rats exposed to low doses of lead (25-300 ppm with postweaning exposures) tended to respond more rapidly than did controls, even though this behavior resulted in withheld rewards. In contrast, animals exposed to higher doses (500 ppm and above) showed decreased rates of FI responding, at least initially. It is hypothesized that the increased response rates may actually be a form of perseverative behavior. The author notes that, in other studies, lead-induced interference with the dopamine system has been suggested as a possible mechanism for perseverative behavior. Also of significance, Cory-Slechta reports that dose-dependent patterns in FI performance, like those in her own study, have consistently been observed across a wide range studies and methodological conditions, including species (i.e., reported effects described in rats, monkeys, sheep, pigeons, and mice) and developmental period (i.e., prenatal, postnatal, postweaning, adult, old adult) during which lead exposure occurs. Thus, evidence in the literature strongly suggests that changes in FI schedule-controlled behavior seem to be one of the most reliable parameters, relative to other measures, for assessing the behavioral effects of experimental lead exposures (Cory-Slechta, 1997).

Rice and Gilbert (1990a, 1990b) conducted a series of behavioral impairment studies on 52 infant monkeys. Shortly after birth monkeys were assigned in equal numbers to one of four feeding groups: (1) a control diet; (2) a lead-containing diet continuously after birth; (3) a lead-containing diet from birth until age 400 days, followed by a control diet; and (4) a control diet from birth until age 300 days, followed by a lead-containing diet. Lead was administered orally in gelatin capsules as lead acetate in 0.05 M sodium carbonate, equivalent to 1.5 mg/kg/day. Feeding regimes were maintained up to and through the time of behavioral testing, which occurred from about 5 through 7 years of age. When monkeys were 5 to 6 years old, they were tested on a series of nonspatial discrimination reversal tasks, including form, form with irrelevant color cues, color with irrelevant form cues, and alternating form and color (Rice and Gilbert, 1990a). Based on this series of tests, the group dosed continuously from birth exhibited the greatest degree of impairment, followed by the group dosed after infancy only. The group dosed during infancy only did not exhibit significant impairment on these tasks. The results of this study provided evidence that while exposure to lead after infancy can produce impairment, this effect is exacerbated if the animal was also exposed during infancy. Approximately one year later, the same group of monkeys were tested on a spatial delayed alternation task in which they were required to alternate responses between two push buttons, with an increasing delay in response time (Rice and

Gilbert, 1990b). In this series of tests, all three exposure groups showed performance impairment relative to controls and were impaired to an approximately equal degree, exhibiting “perseverative behavior” and an inability to suppress inappropriate responses during test delay intervals. The authors conclude that there is not an early critical period for lead-induced impairment on spatial delayed alternation tasks, and that lead exposure only during infancy (i.e., < 400 days) can result in impairment comparable to exposure that continues beyond infancy (Rice and Gilbert, 1990b).

General neurobehavioral effects. A review of animal studies which evaluated the persistence of lead-induced effects on cognitive development was conducted by Tong (1998). Neurobehavioral toxicity was reported to persist for up to 10 years of age in monkeys exposed to low levels of lead and was strongly suggested in some studies to be an irreversible neurotoxin. The physiological evidence of lead disruption of the developing brain (e.g., neuron and synapse formation), and the fact that intracellular lead may not be removable from neural cells, also supports the plausibility of enduring, and possibly irreversible, deficits in neurobehavioral function with lead exposure.

Burger et al. (1998) conducted an experiment on 48 slider turtle hatchlings which were randomly assigned in equal numbers to a control group and three lead exposure groups. The exposed groups experienced a single injection (intramuscularly) of one of three doses of lead acetate (0.25, 1.0, or 2.5 mg/g). No survivors remained in the high dose group by 120 days of age. Behavioral observations, growth (weight and length), and survival data were taken both prior to and at various times after lead injection (i.e., weekly during the first month, and at 4 weeks, 4 months, and 6 months post-exposure). The primary behavioral measure was a righting response (i.e., latency before attempting righting and time required to turn over), as the animals naturally tended to right themselves whenever placed on their backs, and other behavioral measures were not reliably exhibited in this species. By the age of 6 months, righting response adjusted for body weight was significantly impaired by lead dosing. Both the low and medium dose groups performed significantly worse than did controls, and the medium dose group performed significantly worse than did the low dose group, indicating a dose-response effect. The range of lead doses used in the study had such a marked impact on survival that “the threshold for behavioral effects is on the same order of magnitude as the LD₅₀” (i.e., the lowest lead dose at which only 50% of animals would survive). The authors noted that although these results suggest hatchling turtles are vulnerable to lead exposure, behavioral effects due to lead may be very subtle in the field for this species. In addition, when effects do become evident, the levels of lead in turtles may be dangerously close to the threshold for lethality.

USEPA (1986) contains an extensive review of experimental studies using rats exposed to lead. A variety of behavioral and physiological responses affected by lead neurotoxicity, as well as some social interactions in rats, were examined. Although it was generally not possible in the review to standardize results across all studies due to different dose levels and administration modes being utilized across studies at different life stages in the animals, adverse effects were often observed in rats with blood-lead levels around 30 µg/dL, and some effects upon learning were detected even when maximum blood-lead levels were below 20 µg/dL. Many studies (e.g., Angell and Weiss, 1982; Cory-Slechta

and Thompson, 1979; Geist et al., 1985) have observed greater behavioral and physiological effects in rats exposed after weaning or during maturation than in those exposed prenatally or during infancy. Generally lead-exposed rats have been observed to acquire performance skills on discrimination tests more slowly than controls, and to commit more errors.

Social behavior and development. While lead exposure has been linked with the incidence of certain behavioral difficulties in children (e.g., irritation, aggressiveness) since the 1940's, more recent studies have investigated whether such a link exists at low-level lead exposures. One such study investigated the development of social behavior in monkeys and has been documented in Bushnell and Bowman (1979b). In this study, 21 newborn monkeys of both sexes were randomized into three groups of 7 animals each, where the control group received no added lead to their diet, while lead was spiked into the diet of animals in the lower and higher dosed groups over a one-year period in order to achieve targeted average blood-lead concentrations of 50 and 80 µg/dL, respectively. Social tests were administered to the animals once per week for 30-39 weeks, either alone, in the company of the other animals in its group, or with animals of the same age from each group. The frequency and duration of “rough-and-tumble play” was reduced in the lead-exposed groups compared to the control group ($p < 0.05$), while the duration of “contact cling” was significantly higher in these groups. The researchers suggested that although lead-exposed monkeys did occasionally respond to others’ prompts to play, they were less likely to initiate such invitations to others (although their tests did not differentiate between initiating and responding to such play). The lead-exposed monkeys were also observed to be less adaptable to a sudden change in their environment for social interaction (which occurred at approximately 21 weeks of age in this study) compared to control animals.

Bushnell and Bowman (1979b) followed up this experiment with one in which 16 monkeys were placed into four groups of four animals each, where one group served as a control, one was dosed with dietary lead over a one-year period to achieve an average blood-lead concentration of 80 µg/dL (“chronic”), one was dosed with lead for only two weeks at two months of age (“pulse”), and one was dosed under both “chronic” and “pulse” regimens. Animals were tested in a play cage until week 40 (when interactive play behaviors have typically been developed), when they were moved to a play room. This study found that animals exposed chronically to lead had reduced frequency and duration of “rough-and-tumble play,” the development of “initiated social explore,” and the frequency of approach, compared to the control and “pulse” groups. This suggested that reduced desire to play is associated with chronic lead exposure during the period of social development. Meanwhile, animals in the “pulse” group did show significant behavioral effects when the play environment was changed at week 40, suggesting a latent effect associated with an acute exposure early in life and which occurred upon introducing a new and potentially intimidating environment. Furthermore, such effects were less apparent among monkeys exposed to lead only *in utero*.

In a study testing electric shock-elicited aggression (Hastings et al., 1977), lead-exposed animals (through post-natal lead exposure in mother’s milk, where the dams were provided drinking water at concentrations of either 0.02% or 0.20%) showed significantly less aggressive behavior than

did controls. This finding was the opposite of what the authors expected at the time. At 60 days of age (i.e., the time of testing), the lead-exposed animals averaged blood-lead levels of 5-9 µg/dL, and exposure levels were generally low in the study. Other tests performed in this study (e.g., wheel-running, visual discrimination task with reversal) did not see significant differences across exposure groups.

A study (Cutler, 1977) which investigated gender differences with respect to rodent behavior found that lead-exposed male mice showed significantly reduced levels of aggressive behavior (even though body weights and overall activity levels were not affected) compared to controls. In addition, both sexes showed significantly increased social/sexual investigative behavior in the lead-exposed group compared to controls. Total activity and the occurrence of tremors or other jerky movements did not differ significantly between the exposure and control groups.

As cited in Section 2.1.2.1 above, the study performed by Nagymajtenyi et al. (1998) observed an increase in bio-electric aberrations in the brains of rats exposed to lead pre- and post-natally compared to control rats. In addition, behavioral aberrations were observed in this study among the lead-exposed rats. Results showed that lead dosing during pregnancy was related to a significant, dose-dependent increase in hyperactive behavior (e.g., as measured by higher ambulation rates and increased grooming) in the offspring. The authors conclude that low-level lead exposure during prenatal and postnatal development can interfere with normal development and bioelectric functioning within the nervous system and is associated with behavioral changes. In addition, the authors suggest that the functional and behavioral effects of lead, which occur without other overt signs of lead toxicity, are much more harmful than has been previously supposed.

2.1.3 General Health Effects

Although the health effects of lead are diverse, and in general depend on the duration and degree of lead exposure, they are all thought to originate from lead's ability to interfere with fundamental biochemical processes (i.e., mitochondrial energy production, calcium-mediated processes, and protein function). All of the major types of health effects of lead that have been observed in humans, including hematological, neurodevelopmental, immunological, cardiovascular, and renal effects, have been demonstrated in controlled, dose-response studies in rodents, dogs, and/or non-human primates. Although conclusive evidence for carcinogenicity is still lacking in human studies, several animal studies have indicated that there is an association between high levels of oral exposure to several lead compounds and renal tumors in various species (USDHHS, 1999). The U.S. Department of Health and Human Services has determined that lead acetate and lead phosphate may reasonably be expected to be capable of causing cancer, based on sufficient evidence from animal studies (USDHHS, 1999).

While the focus of this report is on the neurobehavioral effects of lead exposure, any type of health effect that is demonstrated to have resulted from lead exposure in animal studies could be of

interest, especially at low lead doses. Therefore, an overview of experimental animal studies which have investigated the general physical health effects that result from lead exposure is presented below. This overview will provide only brief statements of findings across animal studies. Much of the information presented here has previously been cited in USDHHS (1999)¹ and USEPA (1986).

2.1.3.1 Death. At high levels of exposure, lead is known to cause death in humans following severe lead encephalopathy, and has been suggested to be a causative agent in sudden infant death syndrome (USDHHS, 1999). The data are minimal, however, regarding high exposures to lead and death in animals. Increased mortality has been observed in studies with rats and mice given lead in food or drinking water, although in some cases mortality did not occur in a dose-related manner (USDHHS, 1999).

2.1.3.2 Systemic Effects.

Hematological Effects. There have been numerous studies in animals demonstrating the adverse effects of lead on heme (hematin) biosynthesis, which can in turn affect many organ systems. In acute and intermediate-duration studies, the activities of several enzymes involved in the heme biosynthetic pathway have been observed to be altered by administration of lead to rats. Adverse hematological effects have also been observed in rats and dogs in longer term, lower dose studies (USDHHS, 1999).

Renal Effects. The effects of lead on the renal system have been documented in animal studies involving rats, dogs, monkeys, and rabbits. Symptoms of renal insufficiency, including both transient and irreversible kidney lesions, tubular dysfunction, and increased excretion of amino-acids and nitrogen compounds in urine, have been observed at high lead exposures in these studies (USDHHS, 1999; USEPA, 1986).

Cardiovascular Effects. Exposure to lead has been associated with adverse cardiovascular effects in studies with laboratory animals. While older animal studies concluded that hypertension was clearly associated with extremely high doses of lead, the direct effects of lead on blood pressure and the secondary effects (i.e., hypertension as a result of renal damage) of lead were difficult to separate (USEPA, 1986). However, USDHHS (1999) includes a review of several more recent chronic-duration experiments in rats and found that lower lead exposures (i.e., levels that were otherwise non-toxic) were associated with sustained increases in blood pressure as compared to controls. Other adverse cardiovascular effects, such as structural and functional changes relative to controls (e.g., degeneration of the myocardium and aorta), have also been observed in rats following lead ingestion (USDHHS, 1999).

¹ A 1998 draft of this document was also used in obtaining information for this section.

Hepatic Effects. Indications of possible lead toxicity to the hepatic system, as suggested by increased liver weight, morphological changes, and changes in liver enzyme activity, have been observed in animal studies involving mice, rats, dogs, and baboons (USDHHS, 1999; USEPA, 1986). In general, adverse effects of lead on the liver in animal studies were observed at high exposure levels.

Respiratory Effects. There is limited evidence from inhalation studies that prolonged exposure to lead may cause respiratory system irritation in mice. Data suggest that lung irritation, if significant, is largely dependent on the duration of exposure (USDHHS, 1999).

Other Systemic Effects. Lead, at relatively high exposure levels, has also been associated in experimental animal studies with other various systemic effects. These include general impairment of cellular function (via interference with membranes, calcium ion transport, and mitochondrial respiration), long-term visual system deficits in animals exposed during post-natal development, weight loss, impairment of vitamin D metabolism, and endocrine disruption leading to a decrease in thyroid function (USDHHS, 1999; USEPA, 1986).

2.1.3.3 Immunological Effects. The literature provides good evidence that lead can have an immunosuppressive effect on animals at relatively low doses. Although additional research is required to elucidate the exact mechanisms of action, the macrophage is postulated to be the primary immune system target cell for lead (USEPA, 1986). Studies reviewed in USEPA (1986) and USDHHS (1999) showed that in rats, mice, and rabbits, both oral and airborne lead exposures contributed to increased susceptibility to bacterial and viral infections, decreased antibody counts, decreased cell-mediated immunity, depressed lymphocyte function, and suppressed macrophage-dependent immune response, as compared to control animals. In many of the studies cited, lead-induced immunosuppression occurred at low exposure levels that otherwise induced no overt symptoms of systemic lead toxicity, such as increased blood pressure or weight loss.

2.1.3.4 Reproductive and Genotoxic Effects. The observed effects of lead on the reproductive system are mixed across animal studies. Adverse effects observed included decreased pregnancy rates, decreased male fertility, damage to ovaries and testes, and altered pubertal progression, all presumably by interference with hormone production. High doses of lead have also been associated with fetal stunting and fetotoxicity. Lead was not observed to be teratogenic in limited rodent studies, except when lead was administered via injection (USDHHS, 1999). In various animal studies on the genotoxic effects of lead, chronic exposures to lead produced either slight or no significant increases in chromosomal aberrations in mice or monkeys, except in one study (a 1977 study by Deknudt and associates, as cited in USDHHS, 1999) in which monkeys were given a calcium-deficient diet (USDHHS, 1999).

2.1.3.5 Carcinogenicity. Most animal studies conducted to investigate the carcinogenicity of lead compounds have considered only one or two doses, preventing detailed dose-effect characterizations from being performed (USDHHS, 1999). Available data generally suggest that

lead acetate and lead phosphate can be carcinogenic following ingestion by laboratory animals, and that the most common tumor is renal. There are currently no animal data on the carcinogenicity of lead by inhalation or dermal exposure.

In its review of animal studies performed over a 30-year period, USDHHS (1999) has reported that high levels of oral exposure (approximately 25-100 mg/kg/day) to lead acetate and lead phosphate have been shown to increase the incidence of renal tumors in rats and mice (USDHHS, 1999). However, due to the extremely high cumulative doses of lead used in these studies, and the uncertainties regarding mechanisms by which lead induces tumors in the rat kidney (i.e., by acting on species-specific or trans-species proteins), extrapolation to low-level exposure in humans is difficult. Therefore, observations of lead-induced kidney tumors in rats may not be relevant to humans (USDHHS, 1999). The carcinogenicity of lead at lower doses in animals is yet to be determined.

2.1.4 Conclusions from Animal Studies Investigation

Lead has been observed to have widespread neurotoxic effects, as well as behavioral and cognitive symptoms, in humans. These observations are largely consistent with the findings of morphological, electrophysiological, and biochemical studies on animals. Animal studies are also congruent with observations of lead exposure in humans, suggesting an increased susceptibility of the young brain to lead poisoning (Banks et al., 1997). In addition, animal studies have provided physiological evidence that many of the effects of lead on the differentiation of the developing nervous system, such as synaptic and dendritic development, and myelin and other nerve structure formation, have the potential to be long-lived effects (USEPA, 1986). Although animal models do not duplicate the human response to lead exposure, they do serve to provide strong support for expecting certain health effects to occur when humans are exposed. In addition, animal studies allow for more specific determination of dose-response relationships. Single-route exposure and dose-response data are generally not available for humans. Animal studies provide an opportunity to elucidate some of the physiological mechanisms of lead toxicity, and thus they are a valuable tool for assessing the potential risks of lead exposure for human health.

A large resource of published literature also exists in which animal experiments were conducted to investigate the neurobehavioral effects caused by lead dosing. Most of these used monkeys or rats in experiments to assess the effects of lead dosing upon learning and performance activities and were conducted using low to moderate doses of lead. In many studies using monkeys, no other overt signs of toxicity (e.g., weight loss) were observed even when learning impairments were observed.

Several experiments have demonstrated that lead-dosed animals perform similarly to controls on some simple tasks (e.g., visual discrimination of simple shapes), but perform worse than controls on more complex tasks such as reverse discrimination tasks (where the animals have to change a previously-learned strategy) or fixed-interval/delayed response tests. Perseverative behavior is a

recurring phenomenon in the latter type of test, whereby the animal is unable to inhibit inappropriate, repetitious movements despite having rewards withheld as a consequence.

Experiments to compare the severity of impairments in animals dosed only during early development (i.e., prior to birth or during infancy) versus those dosed only during maturation did not yield consistent results. Generalizations about dosing schedules could not be made across all studies or across species, as to which groups of animals fared worse.

There are general similarities in studies and across species, however, with regards to the lowest levels of internal lead exposure during early development at which neurobehavioral effects are commonly observed. For example, the review by Davis et al. (1990) of earlier research findings concluded that internal lead exposures associated with neurobehavioral effects were reported to be as low as $<20 \mu\text{g/dL}$ for rodents, $<15 \mu\text{g/dL}$ for primates, and $10\text{-}15 \mu\text{g/dL}$ for children. Although direct comparisons of blood-lead levels are generally not considered to be accurate across species, evidence that rodents and primates may actually tolerate higher exposures to lead before reaching a given blood-lead level, suggests that exposures and blood-lead levels of concern would even lower in humans than in animals (Davis et al., 1990).

The published literature varied in the amount of detail given to explaining experimental designs used. For example, it was often implied but not always explicitly stated that subjects were randomized to exposure groups according to some statistical criteria, and that the experimenters observing the performance tests were blind to the dosing status of subjects. Articles also provided varying levels of detail on experimental procedures, such as the amount of lead provided through food or water. Blood-lead levels at the time of performance testing were usually measured and reported.

The choice of response endpoint was clearly of particular importance in the design of performance tests. For example, as noted in Burger et al. (1998), behavioral tests need to include procedures in which the given species (absent of lead exposure) will readily participate, otherwise there is a risk that the response endpoint will not be sensitive enough to allow the researcher to detect significant differences between dosed and control animals. Also, lead exposure may delay or accelerate the physical development of some reflexes, so peak performance may occur at different times in different exposure groups. Therefore, the time at which statistical comparisons are made may produce seemingly paradoxical results. In particular, any apparent non-monotonic dose-response relationships must be interpreted with caution.

Despite the aforementioned caveats, animal studies do provide an opportunity to elucidate some of the physiological mechanisms of lead toxicity, as well as generate single-route exposure and dose data that are generally not available in humans. Although animal models cannot claim to duplicate the human response to lead exposure, the resulting ability to control confounding factors permits these models to succeed in providing substantial evidence that supports the existence of a causal relationship between low level lead exposure and neurological impairment, especially in the young.

2.2 SUPPORT FOR THE CAUSALITY OF ADVERSE HEALTH EFFECTS DUE TO LEAD EXPOSURE

Chapter 2 of the §403 risk analysis report documented previously-published information on adverse health effects associated with lead exposure to humans. Subsequent chapters characterized how environmental-lead exposure impacts selected blood-lead concentration and health effect endpoints in children. Specifically, IQ-based health endpoints were selected to represent the neurological effects associated with lead exposure.

A concern, particularly with the IQ-based health endpoints, is whether lead can be assumed to cause the adverse health effects. For ethical reasons, the controlled lead exposure studies necessary to test this hypothesis can not be performed on humans. However, animal studies can be used to supplement the evidence of human studies in this area. To this end, Section 2.1 of this document presented key findings of animal studies that investigated the impact of lead exposure on adverse health effects. This section examines whether the combined evidence of human and animal studies suggests that lead exposure causes neurological damage that can be measured through intelligence testing.

2.2.1 Principles of Causality

On the issue of causality, Needleman & Gatsonis (1990) make the following assertions and present the following principles of causality (citing Kenny, 1979, as a reference):

“Epidemiologic studies cannot, by themselves, establish causal relationships. Causality is not subject to empirical proof, whether in the field or in the laboratory. Given that direct demonstration of proof of a low-dose lead effect in a naturalistic setting is not achievable, epidemiologists rely on canons that, if satisfied, permit the conservative drawing of causal inferences. They are (1) time precedence of the putative cause, (2) biologic plausibility, (3) nonspuriousness, and (4) consistency.”

Needleman & Gatsonis (1990) also introduce a fifth principle, biologic gradient, which is part of biologic plausibility. Needleman (1998) indicates that these principles originate from investigations of whether tobacco use causes cancer and attributes them to the British statistician Sir Austin Bradford Hill. Together, these principles can be applied to the findings of existing studies (both human and animal studies) to imply that lead exposure causes neurological damage that may be measured through IQ score decrements.

The following paragraphs discuss the five principles of causality relative to the evidence in human and animal studies.

Time precedence. Time precedence means that the proposed cause must exist before the proposed effect occurs. That is, a child must become exposed to lead before neurological deficit is observed.

In cross-sectional studies in humans, it is impossible to establish the time precedence of lead exposure and neurological deficit. Longitudinal studies in humans, however, have shown that disturbances in early neurobehavioral development occur even at low lead exposure levels in early life. For example, in Boston, 4 to 8 point differences in performance on the Bayley Mental Development Index were reported at 6, 12, 18, and 24 months, after adjusting for other covariates, when children with low prenatal blood-lead levels (mean of 1.9 µg/dL) were compared to children with modestly elevated prenatal blood-lead levels (mean of 14.6 µg/dL) (Bellinger et al., 1985a, 1985b, 1986a, 1986b, 1987a; 1991; 1992). Additional detail on this and other longitudinal studies is presented in Section 2.2.2 below and Section 2.3.1 of the §403 risk analysis report.

Controlled animal studies further support the time precedence of lead exposure. For example, groups of monkeys exposed to lead either continuously from birth or only after infancy showed learning impairment on a series of non-spatial discrimination reversal tasks, relative to a control group that were not exposed to lead (Rice and Gilbert 1990a). These lead-exposed monkeys, along with a group exposed only during infancy, also showed performance impairment relative to controls in a spatial-delayed alternation task at age 6-7 years. Rats exposed to lead *in utero* and during lactation displayed a highly significant impairment in performance on a water maze test at 100 days of age, relative to controls, even though the average blood-lead level had declined to 1.8 µg/dL by this time (Kuhlman et al., 1997). Additional animal research described in Section 2.1 also supports the conclusion that lead exposure precedes the neurological deficit.

Biologic plausibility. Biologic plausibility means that the causal relationship between exposure and adverse health effects must be consistent with known biological function.

While investigation of the mechanisms of lead toxicity remains the subject of active research, it is known that lead can interfere with cell function by competing with essential minerals, such as calcium and zinc, for binding sites on membranes and proteins. Lead binding to membranes or transport proteins can inhibit or alter ion transport across the membrane or within the cell. In the brain, lead can substitute for calcium and zinc in ion transport events at the synapse (i.e., the junction where the axon of one neuron terminates with the dendrite of another neuron, through which nerve impulses must travel to move from one nerve cell to another). The normally-developing brain appears to delete synapses that are unused and to keep and strengthen synapses that are used. Goldstein (1990, 1992) suggested that lead may disrupt, or delay, the development of synapses and that, perhaps, the resulting connections in the brain are “poorly chosen,” leading to functional impairment. Silbergeld (1991) found that exposure of fetal animals to lead affects both regional growth and synaptogenesis, with synaptogenesis being the more sensitive. Although some of these conclusions are speculative, it is biologically plausible that exposure to lead causes neurological damage.

Biologic gradient. Biologic gradient means that a dose-response relationship must be present (i.e., increased doses of lead should cause increased impairment).

It is well known that high-level exposure to lead produces encephalopathy in children, starting at blood-lead levels of 80 to 100 µg/dL. At lower exposure levels, IQ decrements, fine motor dysfunction, and disturbances in neurobehavioral development have been related to varying levels of lead exposure. These effects are summarized in Section 2.3.1 of the §403 risk analysis report. As summarized in Section 4.4 and Appendix D2 of the §403 risk analysis report, many studies have focused on estimating the magnitude of this dose-response relationship.

Nonspuriousness. Nonspuriousness means that confounding factors associated with adverse health effects must be ruled out.

Studies that investigate the relationship between children's IQ and blood-lead concentration have adjusted for other demographic factors that may affect neurological development, such as Home Observation for Measurement of the Environment (HOME) score, maternal IQ, and socioeconomic status. These studies are summarized in Appendix D2 (Tables D2-1 and D2-2) of the §403 risk analysis report. In many of these studies, the level of lead exposure remains a highly-significant factor after adjusting for these potential confounding factors. Because the method of adjusting for confounding factors in human exposure studies does not necessarily remove all confounding, however, animal research is required to supplement human subject research. Animal studies minimize confounding by administering lead in controlled doses to randomly-selected subjects that are genetically similar and are otherwise treated similarly. Controlled animal studies described in Section 2.1 of this document support the conclusion that lead exposure precedes neurological deficit. In addition, these studies demonstrate that the effects are nonspurious by establishing study designs that include control groups and that attempt to control for confounding factors.

Consistency. Consistency, also known as coherence, requires that the phenomenon be demonstrated in different studies under similar, but not identical conditions.

Human studies investigating the relationship between blood-lead concentration and IQ scores have generally associated IQ decrements with increases in blood-lead concentration in various populations around the world, as summarized in Appendix D2 (Tables D2-1 and D2-2) of the §403 risk analysis report. The magnitude of the estimated IQ decrement varied from study to study, as may be expected, and the relationship was not always statistically significant. However, the relationship was consistently negative (i.e., increased blood-lead concentration was associated with IQ decrement in more than ten studies). Furthermore, a loss of approximately 2-3 IQ points was associated with an increase in blood-lead concentration from 10 to 20 µg/dL in several of these studies.

2.2.2 Causality As Addressed in Longitudinal Studies

The consistency principle of causality implies that causality can not be concluded from the findings of a single human monitoring study. In turn, the time precedence principle indicates that repeated data collection over time for study subjects within longitudinal studies provides an important component of an investigation into the specific role of lead exposure as the cause of certain adverse health effects. Therefore, the findings of multiple longitudinal studies on the association between lead

exposure and diminished performance on cognitive function or intelligence testing can be an important contribution to the argument of causality (while the ability to conclude causality exclusively from such findings remains very limited). The level of importance of the contribution increases when consistent findings are observed across studies (and across different cohorts having different demographic characteristics and lead exposure potential).

This section provides some key findings from two longitudinal studies (Boston and Port Pirie) that can be used in evaluating the hypothesis of causality. This is not meant to be an exhaustive presentation of all results (significant or otherwise) across all longitudinal studies, but instead is a presentation of only key findings from selected studies. For example, results from other longitudinal studies that monitored lead exposure (e.g., Cleveland, Cincinnati, Sydney, Yugoslavia) have not been reviewed. In addition, the potential for reverse causality (i.e., children with lower levels of intelligence are more prone to elevated blood-lead concentrations) is not addressed.

The Boston Prospective Study (Bellinger et al., 1985a, 1985b, 1986a, 1986b, 1987a; 1991; 1992)

The Boston prospective study considered infants born at the Brigham and Women's Hospital in Boston, MA, from August, 1979, to April, 1981. Cord blood-lead levels were measured for 9489 infants (97% of all available infants), and those whose cord blood-lead levels were within one of the following three categories were considered for the study: $< 3 \mu\text{g/dL}$ (low), $6\text{-}7 \mu\text{g/dL}$ (mid), and $\geq 10 \mu\text{g/dL}$ (high). These categories represented the 10th, 50th, and 90th percentiles of the cord blood-lead distribution observed in the first three months of the study. A total of 249 infants in these categories were enrolled in the study (85 in the low category having mean $1.5 \mu\text{g/dL}$, 88 in the mid category having mean $6.5 \mu\text{g/dL}$, and 76 in the high category having mean $14.6 \mu\text{g/dL}$).

The cohort was considered to be of high socioeconomic standing (e.g., 87% of the enrolled infants were white; 92% were considered to come from intact families). While such a cohort tends to have a lower likelihood of lead exposure than more disadvantaged children (e.g., those in poor, inner-city neighborhoods), thereby restricting the ability to generalize results to a wide population, the low occurrence of certain demographic conditions that are typically highly correlated with lead exposure gives this study a greater opportunity to isolate the effect of lead exposure (especially at low levels) on cognitive function.

Post-natal blood-lead concentrations were measured on the study cohort at ages 6, 12, 18, 24, and 57 months, and at age 10 years. Capillary blood was obtained through 24 months of age, and venous blood was obtained at ages 57 months and 10 years. Cognitive function was measured by the Mental Development Index (MDI) of the Bayley Scales of Infant Development at 6, 12, 18, and 24 months of age, by the McCarthy Scales of Children's Abilities at age 57 months, and by the Wechsler Intelligence Scale for Children-Revised (WISC-R) and Battery Composite scores on the Kaufman Test of Educational Achievement -Brief Form (K-TEA) at age 10 years.

Investigations on the association between lead exposure (as measured by cord-lead or blood-lead concentration) and intellectual functioning within the study cohort were performed at various time points during the study. The extent of association was measured by multiple regression modeling, adjusting for parameters that represent potential confounding factors. This resulted in the following key findings:

1. At ages 6, 12, 18, and 24 months, children in the high cord-blood group had significantly lower MDI scores relative to each of the other two groups (4.8 points lower than the low group and 3.8 points lower than the mid group) at the 0.05 level. Furthermore, the MDI scores at these ages were not significantly associated with post-natal blood-lead concentrations measured up to that time (Bellinger et al., 1987). The level of association increased upon adjusting for potential confounders within the multiple regression equation.²
2. By age 57 months, the association between cord-blood grouping and cognitive function diminished considerably from what was observed at earlier ages and was no longer statistically significant, except in the instance where only children with blood-lead concentrations at or above 10 µg/dL at age 57 months was considered. However, a significant (inverse) association was observed between blood-lead concentration at 24 months of age and score on the McCarthy scales at 57 months, upon adjusting for potential confounders³ (Bellinger et al., 1991). The perceptual-performance subscale of the McCarthy scales, which measures visual-spatial and visual-motor integration skills, was especially sensitive to post-natal lead exposure.
3. The association between increased blood-lead concentration at 24 months of age and observed deficits in full-scale and verbal IQ scores was statistically significant at age 10 years, even after adjusting for confounding variables⁴ (p=0.007 for full-scale IQ, 0.004 for verbal IQ, but only 0.091 for performance IQ). Blood-lead concentrations at other ages, however, were not significantly associated with such deficits at the 0.05 level.

² Mother's age, mother's race (white vs. nonwhite), mother's IQ (as measured by the Peabody Picture vocabulary test), mother's education level, # of years mother smoked, # alcoholic drinks per week by mother in the third trimester of pregnancy, Hollingshead Four-Factor Index measure of family social class, quality of care-giving environment, child's sex, child's birth weight, child's gestational age, child's birth order.

³ Family social class, maternal IQ, marital status, preschool attendance, HOME score, # hours per week of "out-of-home" care, # family residence changes, recent medication use, # adults in household, gender, race, birth weight, birth order.

⁴ HOME score at 10 years, total HOME score at 57 months, child stress, maternal age, race, maternal IQ, socioeconomic status, sex, birth order, maternal marital status, and # family residence changes prior to 57 months.

Finding #1 shows that despite a child's lead exposure during the first two years, cognitive function during this period is more significantly associated with pre-natal exposure, which is generally less prone to behavioral and environmental confounding than is direct, post-natal exposure. As a child ages, as seen in Findings #2 and #3, post-natal lead exposure (especially its peak at approximately two years of age) becomes more dominant than pre-natal exposure in its association with the child's performance on intelligence tests. This suggests that the effect of cumulative post-natal exposure through approximately age 2 years eventually outweighs pre-natal exposure. In particular, children can eventually see reduced performance on intelligence testing if their post-natal lead exposure becomes significant, despite their pre-natal lead exposure. Furthermore, a child's post-natal lead exposure tends to change at a faster rate than other demographic variables that are highly correlated with blood-lead levels early in life, with the more recent lead exposures being predictive of a child's current health consequences. These findings support the hypothesis of causality, especially in the relatively homogeneous and highly-privileged cohort considered in this study.

Typically, in environments having high lead levels (e.g., inner-cities, smelter/mining communities), correlations between blood-lead concentrations measured at different ages are so highly correlated that it is difficult to separate out the age effects. However, the lower age-to-age correlations observed in the Boston study (resulting from relatively low lead exposure potential) allowed for investigating age-specific vulnerabilities within this study.

The Port Pirie Cohort Study (Baghurst et al., 1992; Tong et al., 1996; Burns et al., 1999)

This study consisted of 723 subjects born in the lead smelting community of Port Pirie, Australia (and surrounding rural communities) from May, 1979, to May, 1982. Cord-blood was obtained and analyzed for lead (geometric mean = 8.3 $\mu\text{g/dL}$). In addition, capillary blood samples were collected at ages 6 and 15 months and annually from ages 2 through 7 years. A venous blood sample was collected at age 11-13 years. From the measured blood-lead levels, average lifetime blood-lead concentration was calculated for each child using trapezoidal integration. Geometric mean blood-lead concentrations increased to 21.2 $\mu\text{g/dL}$ by age 2, then declined to 7.9 $\mu\text{g/dL}$ by age 11-13, when 375 children remained in the cohort. Children from more advantaged backgrounds were more likely to remain in the cohort through age 11-13 years than children from disadvantaged backgrounds.

Measures of developmental status were made at ages 2 years (Bayley scales), 4 years (McCarthy scales), and at 7 and 11-13 years (Wechsler intelligence scale). In addition, emotional and behavioral problems were assessed by their mothers using a Child Behavior Checklist, and other demographic parameters were measured via questionnaire.

The key findings within this longitudinal study on how lead exposure is associated with decreased developmental status measures were as follows:

1. The inverse relationships between IQ at age 7 years and average lifetime blood-lead concentration measured at 15 months and at 2, 3, and 4 years were statistically significant at the 0.05 level after adjusting for potential confounding factors⁵. This result held for both full-scale and verbal IQ measures, but not performance IQ.
2. Despite blood-lead concentration declining after age 2 or 3 years, the association with cognitive development continued into later childhood. Furthermore, lifetime blood-lead concentration was significantly associated with childhood emotional and behavioral problems (after adjusting for such confounding factors as HOME score, maternal psychopathology, and child's IQ) at ages 11-13 years.

Finding #1 was consistent with the Boston prospective study in that no significant relationship was observed between IQ and pre-natal blood-lead concentration at approximately age 7 years, while the relationship was significant when considering blood-lead concentration at 2 years of age.

2.2.3 Conclusions on Causality

The combined weight of human and animal studies provide evidence, consistent with the principles of causality presented in Section 2.2.1, that lead may be assumed to cause adverse neurological effects in young children. In particular, longitudinal studies in humans have shown that disturbances occur in neurobehavioral development early in life even at low lead exposure levels. These studies have observed effects of lead exposure even after accounting for other demographic factors (e.g., socioeconomic status, parents' IQ) that could affect neurological development. While these other demographic factors tend to be highly correlated with blood-lead levels early in life, the influence that post-natal lead exposure has on blood-lead tends to increase with a child's age. This is because over time, measures of a child's lead exposure tend to change at a faster rate than the child's demographic measures, and more recent lead exposures continue to be predictive of a child's current health consequences. For the §403 risk analysis, adverse neurological effects are assessed through IQ score decrements. Reasons for selecting IQ score decrement as a health effect endpoint are presented in Section 2.5.2 of the §403 risk analysis report.

2.3 THE ASSOCIATION BETWEEN BLOOD-LEAD CONCENTRATION AND IQ SCORE

In its risk characterization, the §403 risk analysis used IQ score decrement associated with lead exposure as the basis for measures of neurological effects. As the risk analysis used blood-lead concentration as its primary measure of body lead burden to quantify environmental-lead exposure, it was necessary to determine IQ score as a function of blood-lead concentration and to characterize the

⁵ Sex, parents' level of education, maternal age at delivery, parents' smoking habits, socioeconomic status, quality of home environment, maternal IQ, birth weight, birth order, feeding method (bottle, breast, both), duration of breast feeding, whether the child's natural parents were living together.

extent to which a change in IQ score occurs when blood-lead concentration changes within a child. The following assumptions were made in this analysis on the association between blood-lead concentration and IQ score for the representative population of 1-2 year old children:

- The relationship between blood-lead concentration and IQ score decrement was assumed to be linear.
- The risk characterization assumed a loss of 0.257 IQ points per 1 $\mu\text{g}/\text{dL}$ increase in blood-lead concentration (with alternatives of 0.185 and 0.323 considered in sensitivity analyses).
- No threshold was assumed in this relationship (i.e., no blood-lead concentration exists below which a relationship between blood-lead concentration and IQ score is not apparent), although selected non-zero thresholds have been assumed in sensitivity analyses presented in Sections 5.1.5 and 6.4.2 of this report.

While the §403 risk analysis report discusses the basis for making these assumptions (e.g., see Section 4.4 and Appendix D2), this section presents additional information that is necessary to judge the correctness and accuracy of the assumptions. Section 2.3.1 addresses the linearity and slope assumptions, while Section 2.3.2 addresses the threshold assumption.

2.3.1 Linearity and Slope Assumptions

How was such an assumption made? As researchers have used primarily linear and log-linear models to characterize the relationship between blood-lead concentration and IQ scores, these two types of models were considered for use in the risk analysis. The log-linear model predicts IQ score as a linear function of log-transformed blood-lead concentration (plus other important confounding variables, such as maternal IQ and HOME score), while the linear model does not take a log transformation of the blood-lead concentration. The scientific community does not appear to have reached a consensus on which form is more appropriate. For example, the meta-analysis in Schwartz (1994) included three studies that employed log-linear models and four studies that employed linear models.

To obtain a single measure of the relationship that would be comparable across studies, despite the different model forms used, Schwartz (1994) used the change in IQ score associated with a doubling of blood-lead concentrations from 10 to 20 $\mu\text{g}/\text{dL}$. The meta-analysis (Schwartz, 1994) yielded an estimated decrease of 2.57 IQ points for an increase in blood-lead concentration from 10 to 20 $\mu\text{g}/\text{dL}$. This was the slope estimate used in the §403 risk analysis.

Using the measure discussed in the previous paragraph, Schwartz (1994) provides some evidence that the log-linear relationship may be more appropriate than the linear relationship. In an analysis to investigate the presence of a threshold (see Section 2.3.2), Schwartz (1994) estimated an IQ point decrement of 3.23 IQ points for the three studies with mean blood-lead concentrations below

15 µg/dL, compared to a 2.32 IQ decrement for the four studies with mean blood-lead concentrations at or above 15 µg/dL. Thus, if anything, a trend toward greater IQ loss associated with lower blood-lead concentrations was observed. This result is consistent with a log-linear relationship.

Despite this evidence, a linear relationship was applied in the §403 risk analysis. The assumption of a linear model reduces the likelihood of overestimating the number of children with low blood-lead concentrations at risk, or who may benefit from actions taken in response to the §403 standards. See Section 4.2.1 and Appendix D2 of the §403 risk analysis report for additional information.

Additional information: Tables D2-1 and D2-2 in Appendix D2 of the §403 risk analysis report summarize a total of 18 studies that report the relationship between children's blood-lead concentration and IQ. Each of these studies was used in at least one of the meta-analysis studies reviewed in Appendix D2 of the §403 risk analysis report. Table 2-2 provides a subset of the information previously reported in Tables D2-1 and D2-2 of the §403 risk analysis report for these studies and also reports the type of model (linear or log-linear) which each study used to predict IQ as a function of blood-lead concentration.

A few of the studies included in Table 2-2 used a log-linear model rather than a linear model to characterize the effect of blood-lead concentration on IQ. However, the overall evidence that these studies provide regarding a log-linear relationship was more limited than the existing evidence on a linear relationship. Furthermore, if EPA had adopted a log-linear model approach, the risk analysis would have estimated that blood-lead concentration had a greater impact on IQ at lower levels than at higher levels. This would have resulted in a greater possibility that the risk analysis would have overestimated benefits at lower levels, compared to underestimating benefits. For these reasons, EPA felt that a linear model was the better approach over a log-linear model. However, the §403 risk analysis did include a sensitivity analysis which considered the effects of a steeper slope in the linear model, in order to evaluate the possibility of underestimating the relationship between blood-lead concentration and IQ.

A recent article by Marais and Wecker (1998) has suggested that researchers who have characterized IQ as a function of blood-lead concentration using linear regression techniques have often reported biased estimates for the effect of blood-lead concentration on IQ for one or both of the following reasons:

- by not having all four of the following predictor variables in the model: blood-lead concentration, mother's intelligence, father's intelligence, and socioeconomic status.
- by not taking into account measurement error in these predictor variables.

Table 2-2. Summary of Key Findings from Studies that Investigate the Relationship Between Blood-Lead Concentration and IQ Score

Study	Type of Study	Location	N	PbB Mean (SD) ($\mu\text{g/dL}$)	IQ Score Mean (SD)	Association Between IQ and Blood-Lead Levels		
						Change in IQ as PbB increases from 10-20 $\mu\text{g/dL}$	P-Value	Model Form
Hatzakis et al. (1987)	Prospective	Lavrion, Greece	509	23.7 (9.2)		-2.7	<0.001	linear
Hatzakis et al. (1989)	Prospective	Lavrion, Greece	509	23.7 (9.2)	87.7 (14.8)	-2.7	<0.001	linear
Bellinger et al. (1991)	Prospective	Boston, MA	150	6.4 (4.1)	115.5 (14.5)	-1.6	0.23	log-linear
Bellinger et al. (1992)	Prospective	Boston, MA	147	6.5 (4.9)	119.1 (14.8)	-5.8	0.007	linear
Baghurst et al. (1992)	Prospective	Port Pirie, Australia	494	20	104.7	-3.3	0.04	log-linear
Ernhart et al. (1989)	Prospective	Cleveland, OH	212	16.7 (6.45)	87.5 (16.6)	-1.1	<0.01	linear
Cooney et al. (1991)	Prospective	Sidney, Australia	175	14.2		+0.4		linear
Schroeder et al. (1985)	Prospective	Wake County, NC	104		Range = 45-140	-2.0	<0.01	linear
Hawk et al. (1986)	Replication of Schroeder Study	Lenoir & New Hanover counties, NC	75	20.9 (9.7)	Range = 59-118	-2.6	<0.05	linear
Dietrich et al. (1993)	Prospective	Cincinnati, OH	231	15.2 (11.3)	86.9 (11.3)	-1.3	<0.10	linear
Yule et al. (1981)	Pilot Study	London, England	166	13.52 (4.13)	98.21 (13.44)	-5.6	0.084	log-linear
Lansdown et al. (1986)	Replication of Yule Study	London, England	166	12.75 (3.07)	105.24 (14.2)	+1.5	0.63	log-linear
Winneke et al. (1990)	Multi-Center, Cross-Sectional Study	Bucharest	301	GM = 18.9 (1.3)			<0.1	linear
		Budapest	254	GM = 18.2 (1.7)			<0.1	linear
		Moden	216	GM = 11.0 (1.3)			<0.1	linear
		Sofia	142	GM = 18.2 (1.6)			<0.1	linear
		Dusseldorf	109	GM = 8.3 (1.4)	116		<0.1	linear
		Dusseldorf	109	7.4 (1.3)			<0.1	linear
Silva (1988)	Cross-Sectional	Dunedin, New Zealand	579	11.1 (4.91)	108.9 (15.12)	-1.5		log-linear
Harvey et al. (1988)	Cross-Sectional	Birmingham, England	177	12.3 (0.2)	105.9 (10.6)			linear
Wang et al. (1989)	Cross-Sectional	Shanghai, China	157	21.1 (10.11)	89	-9		linear
Winneke et al. (1985a)	Cross-Sectional	Nordenham, Germany	122	8.2 (1.4)	120.2 (10.3)		<0.1	linear
Fulton et al. (1987)	Cross-Sectional	Edinburgh, Scotland	501	GM = 11.5	112 (13.4)	-2.6	0.003	log-linear

PbB = blood-lead concentration ($\mu\text{g/dL}$); SD = standard deviation; GM = geometric mean

By analyzing data from four case studies, the authors imply that the bias overestimates the effect, thereby making it likely that the researcher would declare that blood-lead concentration (especially at low levels) has a significant effect on IQ, when in reality, such an effect is insignificant. The authors show how to arrive at an estimate of the blood-lead effect that is not subject to this bias; this estimate is a function of the correlations among the four predictor variables and the measurement variability associated with these variables. The article prompted two responses that were published simultaneously with the article, both of which challenged the article's conclusions.

Despite their claims, the findings in Marais and Wecker (1998) have not resulted in any change to the approach taken by the §403 risk analysis to characterize the relationship between blood-lead concentration and children's IQ for the following reasons:

- Sensitivity analyses performed by other researchers⁶ and documented in a response published with the article by Marais and Wecker (1998) have shown that the approach to obtaining an “unbiased” estimate for the effect of blood-lead on IQ is highly sensitive to the values of the estimates for the correlations and variability among the four predictor variables that are input to the calculation, thereby implying that input values that do not represent the target population can lead to a highly inaccurate estimate for this effect under this approach.
- The authors have not shown that any overestimation associated with this bias is always significantly large enough to warrant concern or will result in an incorrect declaration that blood-lead has a significant effect on IQ.
- The meta-analysis documented in Schwartz (1994), which the §403 risk analysis used to characterize the relationship between blood-lead concentration and IQ, utilizes the estimated blood-lead effects on IQ that were reported by seven studies, six of which estimated these effects after taking into account both parental IQ and socioeconomic status (i.e., the predictor variables of most concern to Marais and Wecker). Furthermore, the §403 risk analysis considered not only the outcome of this meta-analysis, but also the sensitivity analyses associated with this analysis, when investigating the effect of deviation from the meta-analysis outcome on the risk analysis results.
- The need to adjust for measurement error in the predictor variables is not relevant to the §403 risk analysis, as the goal is to predict how a measured blood-lead concentration (after adjusting for the measured values of other potentially important variables) is associated with IQ.

⁶ Waternaux, C., Petkova, E., and DuMouchel, W. “Comment: Problems with Using Auxiliary Information to Correct for Omitted Variables When Estimating the Effect of Lead on IQ.” *Journal of the American Statistical Association*. 93:505-513.

The issue of whether a linear model is appropriate over the entire range of blood-lead concentration must address the presence of a threshold in the relationship. This is discussed further in the next section.

2.3.2 Threshold Assumption

Despite the claims of some researchers on the presence of a threshold in the blood-lead/IQ relationship, the majority of findings across studies and in meta-analyses have failed to find sufficient evidence of a non-zero threshold. Furthermore, when claims of a non-zero threshold were made, the value of this threshold (when suggested) differed considerably across these claims. Therefore, the approach taken in the §403 risk analysis was to assume that no threshold exists (although risk calculations assuming certain non-zero threshold values have been included in sensitivity analyses found in Sections 5.1.5 and 6.4.2 of this document).

In Section 3.3 of USEPA (1998b), the SAB concurred that “available data have not identified a clear threshold,” and, therefore, “the assumption of no threshold for lead effects on IQ score is both defensible and appropriate statistically.” However, it was desired to document the technical justifications for this assumption more thoroughly. Furthermore, the investigation into the presence of a threshold could be addressed by evaluating whether the dose-response function is linear across the entire range of blood-lead concentration.

How was such an assumption made? The assumption of no threshold made in the §403 risk analysis was based on the findings of Schwartz (1994), who noted that the presence of a threshold would result in a decline in the estimated slope associated with blood-lead concentration as the range of blood-lead concentrations declined across studies. However, as mentioned in Section 2.3.1 above, a larger effect size was observed in the four studies with mean blood-lead levels of 15 µg/dL or lower (-0.323 ± 0.126) compared to the other three studies (-0.232 ± 0.040). This observed trend toward higher slopes at lower concentrations discounted the likelihood of a threshold.

Also, Schwartz (1994) examined data from the Boston prospective lead study (discussed in Section 2.2.2 above) specifically to investigate the presence of a threshold. This study was selected as it had the lowest mean blood-lead concentration at two years of age (6.5 µg/dL; n=133) of the studies considered in the meta-analysis, thereby allowing thresholds at low blood-lead levels to be identified if present. In addition, the study cohort’s high socioeconomic (SES) standing may have limited the likelihood of certain confounding, and the Boston study coordinators found relatively weak association between blood-lead concentration at two years of age and various sociodemographic characteristics and psychosocial environment parameters (Bellinger et al., 1986; Bellinger et al., 1992).

Schwartz’s examination of the Boston study data involved fitting two separate regression curves to the same set of covariates: one using IQ score (at 10 years) as the dependent variable, and the other using blood-lead concentration (at 2 years) as the dependent variable. The covariates included age, race, stress and HOME scores, maternal IQ, educational level and occupational status for each parent, mother’s time working out of the house, marital status, gestational age, birth weight, mother’s use of

alcohol during pregnancy, otitis media history, birth order, and SES. Then, a nonparametric smoothed curve (LOESS) was used to characterize the residuals from the IQ score regression as a function of the residuals from the blood-lead regression. The residuals were used in this curve-fitting exercise as they represent blood-lead and IQ score measures after any effects of the above covariates have been removed. The LOESS technique allowed for nonlinear curve fits, such as those that would result if a threshold was present. The curve fit suggested that IQ score decrement was associated with declines in blood-lead concentration even when blood-lead levels were below 5 $\mu\text{g/dL}$, supporting the hypothesis that a blood-lead threshold on IQ score decrement was essentially not present.

In Schwartz (1993), this nonparametric smoothing approach was performed on McCarthy index data collected at age 57 months and blood-lead concentration data collected at 24 months, as recorded in the Boston prospective lead study (Bellinger et al., 1991). Again, after adjusting for potential confounding variables, a definite relationship was observed even at levels below 10 $\mu\text{g/dL}$, with no evidence of a threshold (Schwartz, 1993). To allow any potential threshold to be identified, a piecewise-linear regression model was fitted to these data which allowed the relationship to resemble a “hockey-stick” (i.e., the fit resembled two lines of different slopes that meet at some point representing the potential threshold, with the line below the threshold having nearly a zero slope, and the line above the threshold having a larger, positive slope). This model fit suggested that any potential threshold would be less than 0.0001 $\mu\text{g/dL}$ (Schwartz, 1993).

The meta-analysis by Pocock et al. (1994) involving 26 studies concluded that no single study has collected a sufficient amount of information to make definitive statements on the presence of a threshold, and contradictory results (due to chance) on the presence of a threshold can be observed for different studies. Thus, the analysis did not have enough evidence to reject the hypothesis that no threshold exists.

Identifying a threshold. If a statistical hypothesis is used to determine the presence of a threshold, the test should take the following form:

Null hypothesis: No threshold exists (i.e., the “threshold” is at 0 $\mu\text{g/dL}$)

Alternative hypothesis: A non-zero threshold exists.

If a statistical test is used as a scientific basis for making a decision, one either rejects the null hypothesis or fails to reject it. One never says that the null hypothesis is “true.” Therefore, given a set of data and the statistical methods being applied, one either rejects the hypothesis that no threshold exists or cannot reject it.

The statistical method used to test the above hypotheses can also vary from study to study. In general, the method is applied as part of an investigation into a dose-response relationship between blood-lead concentration and IQ. Two examples of statistical approaches are as follows:

- Several studies (e.g., Dietrich et al., 1993; Hatzakis et al., 1989) investigated dose-response by placing the study cohort into from 5 to 10 groups according to blood-lead concentration, determining the predicted IQ score associated with the mean blood-lead concentration in each group (using some pre-determined regression model), calculating confidence intervals associated with the prediction, and determining how the groups differ in their predictions (as well as any patterns among the groups).
- Another approach focuses on attempting to fit the piecewise-linear “hockey-stick” regression model discussed above that predicts IQ score as a function of blood-lead concentration (and other confounding variables), where the fitted line has a different (larger, positive) slope once blood-lead concentration achieves a certain level, which is interpreted as a threshold value.

Problems associated with suggesting that a threshold exists: Determining whether a threshold exists in the relationship between blood-lead concentration and IQ score is problematic due to the difficulties in accurately characterizing the blood-lead/IQ relationship and the inability to generalize findings across studies and to the nation as a whole. Major sources of these difficulties include the following:

- Different protocols for measuring IQ and different IQ measures (e.g., performance IQ, verbal IQ, full-scale IQ measures are all associated with the Wechsler protocol) are used in different studies.
- Study designs differ, as do the methods used to make inferences from the data.
- Children’s IQ can be difficult to measure and can be more variable than adult IQ.
- Outcomes are often highly dependent on the given set of confounding variables being considered. This set differs from one study to the next. Furthermore, when multiple studies consider the same confounding variables, these variables are often measured differently, using different protocols, from study to study.
- Different ages of children and different ranges of blood-lead concentration are found across studies.

A non-zero threshold would result in reduced estimates of the likelihood of adverse health effects, as children with blood-lead concentrations below the threshold would no longer be labeled as experiencing an exposure-related IQ decrement. The level of reduction would depend on how large the value of the threshold is. Thus, if a decision on a non-zero threshold was made in error, the incidences of adverse health effects would be underestimated. The impact that a non-zero threshold has on reducing the risk estimates calculated in this risk analysis is addressed in sensitivity analyses presented in Sections 5.1.5 and 6.4.2 of this document.

Examples of Possible Non-zero Thresholds Concluded from Study Findings. Relatively high thresholds (e.g., 10 µg/dL or above) have been suggested in some older studies conducted 10 or 15 years ago. However, some of the higher suggested thresholds appear to have lost their legitimacy as they are higher than the levels for which more recent studies have observed some type of health effect. For those older studies that did not report a possible threshold, many involved children with a range of blood-lead concentrations that would be considered high by today's standards. Thus, the findings from these studies cannot be used to determine whether thresholds exist at lower lead levels (i.e., levels below the observed ranges). A study's design must allow for a sufficiently large range of blood-lead concentrations, and in particular, cover a sufficient range of lower-lead levels (i.e., below 5 or 10 µg/dL), to ensure that any threshold value would occur within the observed range.

Some researchers attempting to prove the existence of a threshold have reviewed results of applying the first statistical approach above (i.e., making predictions within groups of the cohort), but have made conclusions based upon simple plots of the results rather than by citing the outcome of statistical comparisons. For example, Kaufman (1996) has concluded that threshold effects may exist at about 20 µg/dL from data presented in Dietrich et al. (1993), at from 10-15 µg/dL from data presented in Bellinger et al. (1992), and at from 25-35 µg/dL in Hatzakis et al. (1989). However, each conclusion was based on visually interpreting selected figures within these articles rather than on the results of controlled statistical hypothesis tests. Furthermore, the following must also be considered when interpreting these conclusions:

- Dietrich et al. (1993): From this prospective study conducted in Cincinnati, OH, Kaufman (1996) cites the authors' presentation of predicted Wechsler Scale Performance IQ (PIQ) for four groups of children approximately 6.5 years of age, where the groups are determined by lifetime mean blood-lead concentration (i.e., average blood-lead concentration measured at 3-month intervals from age 3 to 60 months, and at ages 66 and 72 months). The predicted PIQ score was lower in the group with the highest lifetime mean blood-lead concentration (>20 µg/dL) compared to the other groups. However, the authors caution against interpreting this finding as evidence of a threshold effect, as most children in this group had one or more individual measurements above 30 µg/dL. Furthermore, in his meta-analysis, Schwartz (1994) considered a different relationship cited by the authors: full-scale IQ as a function of average blood-lead concentration through age 3 years (and other covariates, including HOME score and material IQ). This relationship, cited in Appendix D2 of the §403 risk analysis report, is more relevant to the representative population in the risk analysis.
- Bellinger et al. (1992): From this prospective study conducted in Boston, MA, Kaufman (1996) cites the authors' presentation of predicted WISC-R full-scale IQ and K-TEA Battery Composite scores for four groups of 10-year old children, where the groups are determined by blood-lead concentration at 24 months of age (PbB₂₄). Kaufman (1996) indicates that differences were apparent only between the two lowest and the two highest groups, suggesting an apparent threshold between them (i.e., 10-15

µg/dL). However, little difference among any of the four groups would have been identified if the assertion was based on confidence intervals associated with the predictions, rather than standard errors for the individual groups. Meanwhile, the regression model used to predict IQ from PbB₂₄ indicated a highly significant linear trend (p=0.007) across the entire range of observed values of PbB₂₄. This trend was present even among the three groups having the lowest blood-lead concentrations, suggesting that the trend is in fact present at the lower range of the observed concentrations (i.e., below 15 µg/dL).

- Hatzakis et al. (1989): From this study conducted in Greece within a city in which lead mining and smelting occurred, Kaufman (1996) cites how the authors present predicted full-scale IQ for primary school-aged children grouped by blood-lead concentration. Blood-lead concentrations in this study were high: the average blood-lead concentration in this study was 23.7 µg/dL, no child had blood-lead concentration below 7 µg/dL, and more than 90% of the children exceeded 10 µg/dL. The mean predicted IQ in the first two groups (# 14.9 µg/dL, 15-24.9 µg/dL) appeared to be statistically equivalent, then steadily declined for the remaining three groups, suggesting the presence of a threshold around 25 µg/dL. However, many other more recent studies (including animal studies) have observed neurological and developmental effects at lower blood-lead concentrations, making the concept of a threshold at 25 µg/dL highly unlikely. In fact, the linear regression model developed in this study (which included 17 covariates) had a highly significant slope for blood-lead concentration (p < 0.001) across the entire range of data in this study, even though more than 50% of the data occurred from 7-25 µg/dL. Furthermore, it is unclear if different conclusions would have been made if the groups of children were defined differently.

These examples illustrate the complexities associated with characterizing the dose-response relationship and the ability to conclude that a threshold exists in this relationship.

The findings of a study by Fulton et al. (1987), which was included in the Schwartz (1994) meta-analysis, appear to discount the high threshold level suggested by the findings of Hatzakis et al. (1989). This study was conducted on 501 children aged 6-9 years in Scotland. The predicted BASC score was calculated for ten groups of children determined by log-transformed blood-lead concentration. No evidence of a threshold was found among these data, and the estimated slope associated with log-transformed blood-lead concentration was significant (p=0.003). These findings were observed despite having only 10 study children with blood-lead concentrations exceeding 25 µg/dL.

Concluding Possible Thresholds for Tooth-Lead Concentration: Some studies (e.g., Bellinger and Needleman, 1983; Rabinowitz et al., 1992) have observed the potential for thresholds in tooth-lead concentration when relating tooth-lead concentration to IQ score. Based on their investigation of the relation between tooth-lead concentration and IQ score, Rabinowitz et al. (1992)

suggested that a threshold for blood-lead concentration exists at approximately 8 $\mu\text{g}/\text{dL}$. Their investigation was centered around their 1989-1990 study of 764 children in grades 1-3 in Taiwan (an average age of 6.7 years). In this study, teeth shed by these children were analyzed for lead. In addition, the children were administered Raven's Colored Progressive Matrices (CPM) test, the score of which is considered a measure of IQ (average=25, SD=5.7). A model was developed which found CPM test score to be highly correlated with selected non-lead predictors (parental education level, sex, grade level, and whether or not the child is ambidextrous). The difference between the model-predicted and observed test scores for a child (the "CPM score deficit") was interpreted as a measure of the change in the test score that results from lead exposure.

Each of the 380 children for which CPM score deficit could be calculated was placed into one of two groups according to whether or not their tooth-lead level ($\mu\text{g}/\text{g}$) exceeded a specified value. Then, a Mann-Whitney test was performed to determine whether the mean CPM score deficit differed significantly between the two groups. This was done for a series of grouping values for tooth-lead, from 2 to 6 $\mu\text{g}/\text{g}$. Significant differences between the two groups ($p < 0.05$) were seen at grouping levels of tooth-lead at 3.5 $\mu\text{g}/\text{g}$ or above, but not at 3 $\mu\text{g}/\text{g}$ or below. Therefore, the authors concluded that a tooth-lead threshold for intelligence deficit existed at approximately 3.25 $\mu\text{g}/\text{g}$. Finally, the authors relate this tooth-lead threshold value to blood-lead by applying a modeled relationship between tooth-lead and blood-lead levels (formulated from data for 88 Boston children aged 57 months), and concluding that a tooth-lead threshold of 3.25 $\mu\text{g}/\text{g}$ corresponded roughly to a blood-lead threshold of 8 $\mu\text{g}/\text{dL}$ ($\pm 2 \mu\text{g}/\text{dL}$).

While the cohort was considered to have low tooth-lead concentrations, the following must be considered when interpreting the above conclusion on the presence of a threshold and its relevance to the §403 risk analysis:

- A non-zero threshold existing for tooth-lead concentration does not necessarily imply that one exists for blood-lead concentration, as tooth-lead may impact children's health in a different way from blood-lead.
- When noting the lack of significant difference between two groups defined by whether or not tooth-lead exceeds a given threshold when this threshold gets low enough, it is uncertain of the extent to which the lack of significance is actually due to reduced power to detect differences as the sample size in the non-exceedance group declines with the threshold being considered.
- It is uncertain whether the model to predict blood-lead based on tooth-lead from the Boston study, which was used to obtain the blood-lead threshold estimate of 8 $\mu\text{g}/\text{dL}$, can be applied directly to the findings of the Taiwan study without needing to consider certain statistical issues. For example, measurement error associated with tooth-lead levels may differ between the Boston and Taiwan studies.

- Rabinowitz et al. (1992) state that, when attempting to fit a “hockey-stick” regression model to the data, “this data shows no change in the slope (or intercept) of the lines across any trial threshold.” While this statement appears to support the hypothesis that no tooth-lead threshold exists, the authors do not provide any further information on the outcome of this model-based analysis.

2.3.3 Verifying the Results of Schwartz (1994)

Schwartz (1994) applied a random effects modeling approach suggested by DerSimonian and Laird (1986), a highly-regarded reference on meta-analysis. As a result, it was considered among the best encountered by the §403 risk analysis, and therefore, was an important contributor to how the §403 risk analysis characterized the relationship between blood-lead concentration and IQ score in children. For this reason, the meta-analysis findings were verified as part of the §403 risk analysis. Using either the weighted noniterative method or the weighted maximum likelihood method suggested by DerSimonian and Laird (1986), the §403 risk analysis obtained the same finding as Schwartz (1994): that a decrease of 0.257 (\pm 0.041) IQ points was associated with an increase in blood-lead concentration of 1.0 $\mu\text{g}/\text{dL}$ within the range of 10-20 $\mu\text{g}/\text{dL}$. In addition, it was noted that heterogeneity of variance among the seven studies considered by Schwartz (1994) was not significant, and the random effects model gave the same results as a fixed effects model.

2.4 IMPACT OF CERTAIN RESIDENTIAL DUST CHARACTERISTICS ON DUST-LEAD EXPOSURE

The bioavailability of lead can be an important factor in determining the toxic effects of lead exposure to children within a specific environment. Because lead is found in a variety of chemical and physical forms depending on its source, the bioavailability of lead has been studied as a function of chemical make-up (i.e., the particular form of lead present) and particle size in various environmental matrices (e.g., dusts and soils, mining wastes). Generally, the literature concludes that the bioavailability of lead can depend on, among other things, the particular lead species present (which varies depending on the source of lead), the size of the lead-containing particles, the matrix incorporating the lead species, and the types of nutrients or other compounds ingested with the lead (Freeman et al., 1992; USEPA, 1994). It has been suggested that lead speciation and particle size may affect the bioavailability of lead through their influence on solubility (USEPA, 1994). For example, lead bioavailability appears to be lower in mining areas relative to urban and smelter areas. Some authors (e.g., Rieuwerts and Farago, 1995; Davis et al., 1995; Freeman et al., 1992) have suggested that this difference may be, in part, explained by variations in chemical form (dissolutions rates) and particle size. Studies have also shown that correlations between soil-lead and blood-lead levels are influenced by particle size and composition of the lead compounds (USDHHS, 1992).

The purposes of this section are:

1. To present a brief review of the some of the available literature which specifically examines the bioavailability of lead in dust, as a function of particle size and chemical composition of the dust.
2. To determine if there is evidence which warrants the consideration of particle size and lead speciation, as related to lead bioavailability in dust, in the §403 rulemaking; and, if warranted, determine if there is sufficient information available in the literature to allow for a thorough consideration.
3. If relevant, to identify significant information gaps and potential issues that may warrant further research.

2.4.1 Review of Literature: Effects of Chemical Composition on Lead Bioavailability in Dust

There is substantial evidence in the scientific literature that the particular chemical species, as well as the matrix (e.g., mineralogy, organic matter content) within which the lead compound is found, are important in determining lead bioavailability (USDHHS, 1999; USEPA, 1994). Many of the studies in the literature have been based on comparisons of relatively simple lead compounds in controlled animal feeding studies or have focused on lead bioavailability in urban and mining-associated soils. With respect to household dust in particular, there is relatively little in the literature which specifically examines the relationship between bioavailability and chemical composition.

The literature does recognize, however, that the composition of interior dust is substantially influenced by soil and exterior dust (Diemel et al., 1981; USEPA, 1994). For example, USEPA (1994) characterizes total lead in household dust as being comprised of soil-lead, air-lead, lead from outside sources (e.g., workplace, school), and lead from household paint. As a default value to the IEUBK exposure model, EPA has set the ratio of household dust-lead concentration to soil-lead concentration at 0.70, which was considered appropriate for neighborhoods or residences where loose particles of surface soil are readily transported into the house (USEPA, 1994). Thus, soil particles have the potential to be a significant contributor to lead levels in household dust.

The following sections will provide a brief review of scientific findings related to the general physical and chemical principles of lead and how they relate to bioavailability differences in controlled environments and in soil studies. Because the literature recognizes that the composition of interior dust is influenced by soil and exterior dust, discussion of these general bioavailability factors will, in the absence of more dust-specific data, serve as a starting point in understanding the relationship between lead bioavailability in household dust and chemical composition and particle size.

2.4.1.1 Research on Lead Bioavailability in Controlled Animal Studies. The relative bioavailabilities of simple lead compounds have been studied under controlled conditions in animal studies. For example, Barltrop and Meek (1975) (as cited in USDHHS, 1992) compared the absorptions of 12 different lead compounds in rats by measuring the kidney contents following oral

exposure. They found that the absorption of metallic-lead (particle size 180-250 Fm) was the lowest of the lead compounds tested. Data also suggested that the absorption of lead sulfide (particle size <50 Fm) was significantly less than the oral bioavailability of other lead salts (oxide, acetate). Lead carbonate had the highest absorption, which was suggested to be due to its high solubility in gastric juice.

Dieter et al. (1993) also found differing blood-lead, bone-lead and kidney-lead levels in rats fed different lead compounds, indicating variability in bioavailability. For example, maximum blood-lead levels were higher (80 Fg/dl) in rats fed lead acetate and lead oxide, in comparison to rats fed lead sulfide and a lead ore concentrate. Similar differences were observed in bone-lead and kidney-lead levels between the rats receiving the more soluble (e.g., lead acetate and oxide) and less soluble (e.g., lead sulfide and ore) lead compounds.

2.4.1.2 Research on Lead Bioavailability in Soils. The literature also suggests that the soil matrix itself can be an important factor in determining the bioavailability of lead. For example, Freeman et al. (1992) found that tissue-lead concentrations were lower in rats fed lead-contaminated mining waste soils from Butte, Montana, as compared to rats fed comparable doses of soluble lead acetate. It was suggested that the inherent chemical properties of soil-adsorption sites and the alteration of lead-bearing solids (e.g., encapsulation processes which inhibit dissolution) may reduce the bioavailability of soil-lead, as compared to lead ingested without soil. In general, the fate and bioavailability of lead in soils are affected by the species of lead incorporated into the soil, the degree of absorption at mineral interfaces, precipitation of solid phases, and the formation of relatively stable complexes/chelates with organic matter, as well as other complex soil matrix factors such as pH (USDHHS, 1999; McKinney, 1993; Freeman et al., 1992). This has been suggested to be largely due to the influence of these factors on solubility, although it is important to note that solubility is but one factor in the bioavailability of lead to humans or animals (USEPA, 1994).

In a study of lead bioavailability in soil, Laperche et al. (1997) found that apatite (calcium fluoride phosphate) amendments to a lead-contaminated soil lowered the bioavailability of soil lead (as determined by plant uptake) by inducing the formation of geochemically-stable lead phosphate compounds. Similarly, in a study of an old mining village with elevated lead levels in both garden soils and house dust, Cotter-Howells (1994) identified the predominance of lead phosphate compounds (of limited bioavailability) as probable explanation of why blood-lead levels in the village were not elevated in an otherwise contaminated area. In a study of mining-associated soils in Butte, Montana, Davis et al. (1995) suggested that the predominance of lead sulfide/sulfate and oxide/phosphates in soil and mine waste samples might provide an explanation for the limited lead bioavailability that was observed when the Butte soils were fed to rats in a previous study (Freeman et al., 1992).

Urban area soils are typically contaminated with alkyl lead species originating from combustion of leaded gasoline; lead halides (chlorides and bromides) from auto exhaust particulates; or lead carbonate, chromate, and octoate (as chips, flakes, and dusts) from exterior and interior lead-based paint (USEPA, 1994). Lead halides in soils are quickly transformed to (or associated with) oxides or

sulfates (USEPA, 1986 as cited in USEPA, 1994). In many lead-mining districts, the predominant form of lead is galena or lead sulfide (USDHHS, 1992).

2.4.2 Review of Literature: Effects of Particle Size on Lead Bioavailability in Dust

Data in the literature are limited with specific regards to how particle size of lead-contaminated house dust influences the bioavailability of lead in the dust. However, studies have been conducted to examine the general relationship in soils between particle size and bioavailability, as well as particle size and lead concentration. For example, Barltrop and Meek (1979) found that the bioavailability of lead in the intestinal tract of rats fed metallic-lead of various particle sizes increased fivefold as particle size decreased from 197 microns to 6 microns. Particle size, due to kinetic limitations that control dissolution rates in the gastrointestinal tract, has also been hypothesized to contribute to the lower bioavailability of lead observed in mining waste soils relative to urban and smelter soils (Davis et al., 1995). In general, the smaller the particle size, the greater the absorption of lead due to more rapid dissolution (small particles have higher surface area to mass) in the gastrointestinal tract (Freeman et al., 1992).

Que Hee et al. (1985) found that when lead concentrations were measured in dust samples categorized by size fraction, lead concentration was generally independent of the particle size. However, most of the dust particle mass (about 75%), and thus most of the lead (about 77%), was present in the <149 Fm size fraction. Lead concentration in smaller particle size ranges may possibly maximize intestinal absorption, and thus increase bioavailability (USDHHS, 1992). Duggan and Inskip (1985) performed an extensive literature review on the variation of lead concentration with particle size and reported that higher lead concentrations are usually found in the smaller-sized fractions of soil and dust. As reported by the Agency for Toxic Substances and Disease Registry, numerous studies have also observed the lead content of soil, street dust, city dust, and house dust to increase with decreasing particle size (USDHHS, 1992).

2.4.3 Information Gaps, Issues and Conclusions

Although the literature is generally lacking in data which directly address the bioavailability of lead in household dust as a function of chemical composition and particle size, by recognizing that interior dust composition can be greatly influenced by outside soil, it can reasonably be expected that the factors which affect lead bioavailability in soils will also influence the bioavailability of lead in household dust. Therefore, based on general knowledge of the bioavailability of simple lead compounds and studies of lead compounds in soil matrices, evidence suggests that particle size and chemical composition have the potential to significantly affect lead bioavailability in dust. Nonetheless, the current information base which specifically addresses particle size and chemical composition of dust as factors in lead bioavailability may be inadequate to determine how such factors can reasonably be incorporated into the rulemaking effort. Furthermore, needing to characterize dust by particle size and lead by chemical speciation within a risk assessment will likely add to the expense of dust analyses, and dust standards that distinguish between these various characterizations could add considerable complexity to the rule.

Specific uncertainties that remain concerning bioavailability of lead in household dust include the following: physical and chemical properties that may be unique to dust versus soil; whether the effect of lead speciation in dust is significant enough to affect dust standards for lead; distributions of lead across particle sizes found in household dust (e.g., whether dust is enriched with the smaller size fraction relative to outside soil) and whether particle size differences are significant enough to affect standards; and possibly variances in exposure mechanisms that may occur across particle sizes.

The need for further research in these and related areas has been supported by several authors. For example, Freeman et al. (1992), based on comparisons of mining waste soils and other soil types in reviewed studies, emphasized the importance of evaluating the soil mineralogy and lead species present when predicting bioavailability values for lead in soils. In addition, USEPA (1994) in the *Guidance Manual for the Integrated Exposure Uptake Biokinetic Model for Lead in Children*, notes that adequate characterization of lead contaminated media, for the purpose of estimating bioavailability, should include assessment of physical and chemical parameters, such as particle size and media solubility.