Toxicological Review and Recommended Toxicological Reference Values for Environmental Lead Exposure in Canada

Prepared for:

Health Canada

Healthy Environments and Consumer Safety Branch Safe Environments Directorate Environmental Health Bureau Contaminated Sites Division Ottawa, ON

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Note to Readers

Units

The SI units for the concentration of lead in blood are μ mol/L. Most studies, however, report blood lead concentrations in units of μ g/dL. By convention, blood lead concentrations are presented in this report in units of μ g/dL. To convert blood lead concentrations in μ g/dL to μ mol/L, divide by 20.72.

This report was prepared under contract to Health Canada's Contaminated Sites Division; however, this report does not necessarily reflect the opinion of Health Canada nor is it Health Canada guidance.

Authorship & History of this Report

Norm Healey is the first author and overall editor of this report. *Heather Jones-Otazo* researched and authored the review of epidemiological evidence of the carcinogenic effects of lead.

Mike Walker used literature reported cancer bioassay data to conduct cancer doseresponse modeling and calculate quantitative estimates of cancer potency. *Anthony Knafla* is the first author of a contract report on the toxicology of lead that was previously commissioned by Health Canada (Equilibrium Environmental Inc., 2008a). The review of the toxicological literature on lead in this report is based partly on Equilibrium Environmental Inc.(2008a) and Anthony Knafla is the first author of the analysis of population health effects associated with a decrement in mean IQ of one point and an increase in mean systolic blood pressure of 1.3 mmHg (Appendix A).

The first draft of this report (December 2008) was compiled and subject to international peer review while Norm Healey was employed by Health Canada. Norm Healey resigned from Health Canada and began working for Azimuth Consulting Group Inc. (Azimuth) in February 2009. Azimuth, under subcontract to Equilibrium Environmental Inc., was contracted during the 2009-2010 fiscal year to update the December 2008 draft report in response to referee comments.

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Extended Summary

Introduction

This report presents a critical review of the evidence of the toxicological effects of lead (Pb) and recommends toxicological reference values (TRVs) for primary prevention of adverse population health effects from environmental lead exposure in Canada.

The lead TRVs presented in this report are not intended to define the maximum amount of environmental lead exposure that is *tolerable* or *acceptable*. The lead TRVs presented in this report are points of *reference* to help risk assessors quantify the potential health risks associated with a given lead exposure scenario and describe the associated uncertainty. Whether or not the potential health risks are *tolerable* or *acceptable* is a value judgement that is within the purview of risk managers and stakeholders.

This report has three general parts: (1) an overview of the diverse systemic toxic effects of lead and a comparison of the relative weight of evidence for adverse effects on specific systems or endpoints at relatively low blood lead concentrations; (2) detailed reviews of the evidence for three "critical" effects: developmental neurotoxicity, toxicity to the vascular system, and cancer; and (3) the derivation of TRVs for these critical effects.

TRVs for non-cancer effects have traditionally been based on the assumption that a threshold of toxicity can be identified and that the TRV is less than this putative threshold. In the case of lead, however, no threshold can currently be identified for developmental neurotoxicity, vascular toxicity and several other systemic effects.

Additionally, the US National Research Council, several other publications by researchers and risk assessors, and some of the referees of an earlier draft of this report recommend that TRVs for non-carcinogens, and lead in particular, be based on the assumption that there is no threshold for population health effects. Under this alternative framework, the slope of the dose-response relationship is estimated and a probabilistic, risk-specific dose is calculated from this slope. Accordingly, this report provides recommended slopes for the relationship between blood lead concentrations and IQ deficits in children and for the relationship between blood lead concentrations and increased systolic blood pressure (SBP) in adults. These slopes are extrapolated to the origin to derive risk-specific blood lead concentrations for population effects on IQ and SBP.

Background

History of Parallel Declines in Lead Exposure and Reference Values

There are no known physiological requirements for or functions of lead (i.e., lead is not an essential element). Current blood lead concentrations are estimated to be at least 100-fold higher than natural background levels (i.e. blood lead concentrations of humans prior to mining and smelting lead) (Ericson *et al.*, 1979; Ericson *et al.*, 1991; Wittmers *et al.*, 2008).

Average blood lead concentrations in western nations, including Canada, have declined by about 10-fold since the 1970s. As blood lead concentrations have declined scientists have been able to study the potential toxic effects of lead at ever decreasing concentrations. This has resulted in a continued history of "discovering"

that lead exposure levels that were previously thought to be safe, or without risk of harm, are associated with adverse health effects.

As blood lead concentrations and estimates of toxic thresholds have declined, so too has government policy on what constitutes a maximally tolerable lead intake and blood lead concentration. The US blood lead intervention level declined six-fold from 1970 to 1991, but has remained constant since. A similar history of declines has occurred in Canada.

The maximum daily intake of lead from all sources recommended by an expert committee of the US Public Health Service in 1971 was 300 μ g/d (equivalent to about 20 μ g/kg/d for a toddler) (King, 1971). At the time, it was thought that intakes less than this amount would not result in the accumulation of lead in a child's body. In 1986 the World Health Organization and Food and Agriculture Organization (WHO/FAO) Joint Expert Committee on Food Additives (JECFA) established a provisional tolerable weekly intake (pTWI) of 25 μ g/kg (equivalent to 3.6 μ g/kg/d) also based on the understanding that this level of intake would not result in the accumulation of lead in a child's body. In 1999 JECFA upheld this pTWI based on the understanding that it would not produce a blood lead concentration in children greater than 10 μ g/dL.

Health Canada's existing TRV for lead is a provisional tolerable daily intake (pTDI) of 3.6 µg/kg/d and this value is based on JECFA's pTWI. The pTDI was originally derived to prevent an unacceptable accumulation of lead in blood. However, recent advances in the understanding of the toxicokinetics of lead means that there is considerable uncertainty in the blood and bone lead concentrations associated with chronic ingestion of lead at this dose. Also, recent epidemiological and toxicological

literature on the health effects of lead suggests that blood lead concentrations that were previously considered safe may be associated with risk of adverse health effects. Taken together, these developments raise uncertainties about the level of health protection that is provided by Health Canada's existing pTDI for lead.

Distinguishing TRVs for Prevention from TRVs for Blood Lead Intervention Values

The TRVs for lead derived in this report are not synonymous with blood lead intervention values and are not intended to replace them.

The TRVs for lead presented in this report are developed for primary prevention purposes and they are distinct from blood lead intervention values. The TRVs derived in this report are provided to guide decision-making about *prevention* of population level health effects from exposure to lead in the environment. Blood lead intervention levels, on the other hand, are used to guide decision-making about *intervention* after exposure has already occurred – most often on an individual basis in a clinical setting. This requires balancing the probability of significant toxicological effects on an individual basis against the efficacy and costs of individual level medical or environmental intervention. The TRVs for lead presented in this report should not be used to interpret the toxicological consequences of lead exposure on an individual basis. Health Canada's blood lead intervention values are currently under review.

Metric of Lead Exposure

In much of the literature relating lead exposure to health effects, exposure is expressed as a biomarker concentration - most commonly blood lead and more

recently bone lead. For this reason, the health effects of lead are better understood in the context of associated blood or bone lead concentrations, rather than external dose or environmental concentrations. Therefore, the focus of this report is on describing the relationship between blood lead concentrations, and to a lesser extent bone lead concentrations, and health effects. An exception to this is the animal cancer assays, where lead exposure has been primarily measured as an oral dose, rather than biomarker concentration. The TRVs for cancer presented in this report are expressed as oral doses. The TRVs for non-cancer endpoints are expressed in blood lead concentrations.

Methods

Critical Effects

A TRV is derived on the basis of a quantified dose-response relationship for a single health endpoint. However, a TRV is also intended to protect against all adverse health effects associated with a substance. Therefore, it is important to examine all of the potential adverse effects of a substance and ensure that the TRV is derived on the basis of the critical, or most sensitive, effect. A critical effect is defined as the first biologically significant adverse effect expected to occur as exposure dose or concentration increases above zero (Health Canada, 1994; US EPA, 2007b).

A critical effect by this definition cannot be identified for lead, however, because many of the systematic effects of lead are without an identified threshold. Instead, the critical effects for lead are defined as: (1) those endpoints or effects that have the greatest relative weight of evidence at the lowest blood lead concentrations; and (2) those outcomes for which a blood lead concentration-response relationship can be characterized with the greatest relative certainty.

Tiered Approach to the Review of Evidence

The review of toxicological evidence followed a tiered approach. This was done as a matter of efficiency.

Tier I Evidence for Identification of Critical Effects

As a first tier, third party reviews were consulted to develop a general understanding of the blood lead concentration-response relationships for each of the major biological systems or organs affected by lead. Third party reviews that were consulted for this evidence included peer reviewed contractor reports commissioned by Health Canada, recently published peer reviewed toxicological reviews of lead by other health agencies, and recent review articles published in the peer reviewed literature. Original sources were obtained and reviewed where clarification or confirmation was required.

Tier II Evidence from the Systematic Review of Critical Effects

A systematic search of the available English language literature was conducted for each of the identified critical effects. Primary searches were conducted in the MEDLINE/*PubMed* database using relevant MeSH¹ terms and the TOXLINE database using key words. The results of the database searches were verified by cross referencing literature cited in recently published review articles. The cut-off publication date for systematic searches was December 31, 2007. However, current

¹ MeSH is the U.S. National Library of Medicine's controlled vocabulary used for indexing articles for the MEDLINE/*PubMed* database.

awareness was maintained by using Ovid Current Content alerts and this report includes any relevant papers published before October 2009.

Non-threshold TRVs for Non-cancer Effects of Lead

The report provides recommended slopes for the relationship between blood lead concentrations and IQ deficits in children and for the relationship between blood lead concentrations and increased systolic blood pressure (SBP) in adults. These slopes are extrapolated to the origin to derive risk-specific blood lead concentrations for population effects on IQ and SBP.

Risk assessors can use the recommended dose-response slopes for critical effects to quantify the health risks associated with different lead exposure scenarios. This approach offers advantages over the traditional TRVs for non-carcinogens, which only allowed non-cancer risks to be characterized qualitatively as "acceptable" or "unacceptable".

The recommended slopes were used to calculate risk-specific blood lead concentrations for a specified level of population health risk. These risk-specific blood lead concentrations were calculated to provide a convenient point of reference for risk assessors, risk managers, and stakeholders. They are not intended to define maximum acceptable or tolerable exposures. The methods presented in this report can be used to calculate blood lead concentrations associated with alternate levels of population health risks for the critical endpoints.

Blood Lead Concentrations and Health Effects

The evidence of the toxic effects of lead, as measured by blood lead concentrations, on various organs and systems was reviewed. There is, to varying degrees, some evidence of adverse effects on all organ systems down to the lowest blood lead concentrations studied.

The wide variety of tissues and systems adversely affected by lead and the complexity of lead toxicity may be explained by the presence of some common underlying modes of toxicity that have the potential to effect the functioning of many different cell types. These potential mechanisms include oxidative and nitrosative stress, Ca²⁺ and Zn²⁺ mimicry, and sulphydryl (thiol group) binding. Some of these modes of toxicity are also common to other metals, such as mercury and cadmium.

Some of the modes of toxicity associated with lead, such as oxidative and nitrosative stress and altered DNA methylation and associated gene expression, also contribute to the pathogenesis of diseases with multiple environmental and hereditary risk factors, such as atherosclerosis, hypertension, neurodegenerative disease, renal dysfunction, and cancer. In this context, the adverse effects associated with environmental lead exposures can be thought of as an additional risk factor that can exacerbate and accelerate the onset of these diseases.

The following systems have at least moderately strong evidence of adverse effects associated with chronic blood lead concentrations < 10 μ g/dL:

- Neurological
- Cardiovascular
- Reproductive
- Haematopoietic
- Immune
- Renal

Moderately strong evidence of adverse effects is defined as: (1) multiple epidemiological studies that are predominantly positive for an effect within this range, or (2) a single good quality animal study that is positive for an effect within this range.

The critical effects for lead are defined as: (1) those endpoints or systematic effects that have the greatest relative weight of evidence at the lowest blood lead concentrations; and (2) those outcomes for which a blood lead concentration-response relationship can be characterized with the greatest relative certainty. Of the six systems for which there is at least a moderately strong weight of evidence of adverse effects at blood lead concentrations less than 10 μ g/dL, developmental neurotoxicity and cardiovascular toxicity best meet these criteria. These endpoints were carried forward as candidate critical effects.

Cancer was also identified as a candidate critical effect, although the weight of evidence for carcinogenic effects at environmentally relevant blood lead concentrations is relatively weak. However, in 2004 the International Agency for Research on Cancer (IARC) re-classified inorganic lead compounds as Group 2A Carcinogens (probably carcinogenic to humans) (IARC, 2006). Health Canada derives quantitative estimates of carcinogenic potency for substances classified under the Canadian Environmental Protection Act (CEPA) classification scheme as Group I or II Carcinogens (Health Canada, 1994; Health Canada, 1995; Health Canada, 1996). The criteria for a CEPA Group II Carcinogen is very similar to the criteria for an IARC 2A carcinogen. Therefore, cancer was identified as a potential critical endpoint and was carried forward for evaluation under the CEPA classification scheme for carcinogens. It is Health Canada policy to derive a quantitative estimate of carcinogenic potency for CEPA Group I and II Carcinogens, but substances are ultimately managed on the basis of their most sensitive effect (carcinogenic or non-carcinogenic). More detailed reviews of the observational and experimental evidence of effects were undertaken for the candidate critical effects: developmental neurotoxicity, cardiovascular toxicity, and cancer. It is emphasized that these are not the only endpoints for which there is evidence of adverse effects associated with relatively low blood lead concentrations. Risk assessors should keep in mind that these endpoints, to a degree, are surrogates for all of the other potential health effects associated with a given blood lead concentration.

Developmental Neurotoxicity

There are multiple lines of evidence, including human epidemiological studies, *in vivo* animal assays, and *in vitro* experiments, that support the hypothesis that chronic lead exposure can result in developmental neurotoxicity in humans.

Lead exposure has been associated with adverse developmental effects on a variety neurological endpoints including:

- Neuromotor function (Dietrich et al., 1993a; Wasserman et al., 2000b; Ris et al., 2004; Despres et al., 2005; Fraser et al., 2006)
- Academic achievement (Needleman et al., 1990; Fergusson et al., 1997; Al-Saleh et al., 2001; Wang et al., 2002; Miranda et al., 2006)
- Delinquent or antisocial behaviour (Fergusson et al., 1993; Bellinger et al., 1994b; Needleman et al., 1996; Dietrich et al., 2001; Needleman et al., 2002)
- Attention and exectutive function (Bellinger et al., 1994a; Canfield et al., 2003b; Chiodo et al., 2004; Ris et al., 2004; Braun et al., 2006; Chiodo et al., 2007)
- Auditory function (Schwartz and Otto, 1991; Dietrich et al., 1992; Osman et al., 1999)

 Visual function (Rothenberg et al., 2002b; Fox *et al.*, 1997; Fox *et al.*, 2008; Laughlin *et al.*, 2008)

The potential for lead to affect scores on psychometric tests of intelligence (IQ) among school-aged children was selected as the critical endpoint for derivation of a TRV for the developmental neurotoxicity of lead. IQ was selected as the critical developmental endpoint because, of all the developmental neurotoxicity endpoints that have been examined, IQ has the greatest weight of evidence of adverse effect at relatively low blood lead concentrations. IQ has also been reliably related to latter life outcomes, such as academic achievement and earning potential. It is important to keep this point in mind when contemplating the implications of the potential effects of lead exposure on IQ of children: IQ is a surrogate for many other neurological outcomes and adverse consequences beyond the immediate implications of reduced performance on psychometric tests of intelligence.

Epidemiological Evidence of Developmental Neurotoxicity

The epidemiological evidence is strongly suggestive, but not consistently supportive, of an association between early life chronic lead exposure (as measured by various biomarkers) and decrements in school-aged children's IQ, after correcting for covariates. This association has been demonstrated in a wide variety of ethnic and socioeconomic populations across a wide range of lead exposures.

Most, but not all, epidemiological studies of early life lead exposure and IQ have reported an inverse association. Not always has this association reached statistical significance after adjusting for potential confounding. Rarely have studies indicated that the association is in the opposite direction. Given the potential insensitivity of IQ as a measure of neurological injury, the potential imprecision of blood or bone lead as a measure of exposure, the complex and multi-factorial determinants of neurological development, and the possibility that some of the variables that are treated as confounders may also be effect modifiers, the consistency with which early life lead exposure has been associated with IQ decrements should be interpreted as a sign of the strength of the association. The few null findings should be expected given the complexity of the relationship under study and the limited sensitivity of the tools. As a point of comparison, a significant inverse association between early life blood lead concentration and IQ, after adjusting for confounders, has been more consistently reported than a significant positive association between sodium intake and blood pressure (Institute of Medicine, 2005).

As of January 1st 2008 there were 12 published longitudinal studies of early life lead exposure and potential effects on psychometric test scores and also a pooled analysis of the data from seven of the eight longitudinal studies that had been initiated prior to 1995. Four of the 12 longitudinal studies and the international pooled analysis are strongly supportive of an association between early life lead exposure and potential effects on psychometric test scores. Six of the 12 longitudinal studies report inconsistent results across biomarker of lead exposure, time point of exposure or assessment, or method of measuring IQ. Two of the 12 longitudinal studies consistently failed to find a significant association.

The pattern of results does not appear to be dependent on cohort demographics, such as SES, nor do they appear to be dependent on exposure range – significant associations have been reported among both relatively low and relatively high socioeconomic strata as well as across relatively low and relatively high blood lead concentrations. One pattern to the results of the longitudinal studies is that the three studies with the most subjects were all strongly positive. These were the Treatment of Lead Exposed Children (TLC), Port Pirie and Kosovo cohorts. The results of the

international pooled analysis are also consistent with this pattern. The results of the analysis of data from a combined 1,303 subjects were consistently and strongly positive for a significant inverse association between children's blood lead concentrations and corrected IQ. The one exception to this pattern is the results from the Rochester cohort, which were also consistently and strongly positive, but were observed in an analysis with relatively few (~170) subjects.

Four meta-analyses published since 1990 on the relationship between maternal or postnatal blood lead and psychometric tests of intelligence were identified and reviewed for this report. The meta-analyses were unanimous in their conclusions that the available epidemiological evidence supports an inverse association between childhood blood lead and IQ over the range of lead exposures captured by the constituent studies.

There is evidence that the developmental neurotoxic effects associated with childhood blood lead concentrations persist out to at least the late teen-age years. Early life lead exposure, as measured by blood lead concentrations, have been associated with deficits in academic achievement and psychometric tests of intelligence out to at least 17 years old. No threshold for these effects has yet been identified and there is evidence that the biomarker-response relationship extends as low as the minimum range of blood lead concentrations examined in the existing literature. There is also evidence of a steeper biomarker-response relationship over the lower range of studied blood lead concentrations.

Bone Lead and Developmental Neurotoxicity

Only one study that examines the relationship between children's bone lead and IQ was identified at the time of this report - that from the Yugoslavia cohort

(Wasserman *et al.*, 2003). This study reported a significant inverse relationship between tibia lead measured at 10-13 years old and IQ measured at 10-12 years old. Bellinger *et al.* (1994a) reported a significant inverse relationship between tibia and patella lead and neurophsychological tests of attention (Mirsky battery) among 79 young adults (19-20 years old). Needleman *et al.* (1996) also reported a significant association between tibia lead measured at 12 years and antisocial and aggressive behaviour as rated by teacher and parents on the Child Behaviour Checklist (CBCL). Gomaa *et al.* (2002) also reported an association between maternal patella lead, but not tibia lead and neurodevelopment in 2 year olds from Mexico City.

Experimental Evidence of Developmental Neurotoxicity

Laboratory Animals

There is strong evidence of central nervous system (CNS) damage in lead exposed animals from a wide variety of endpoints, including biochemical markers of effect, pathological disturbances, and functional impairments. Animal studies demonstrate lead-induced adverse effects on auditory function (Rice, 1997); visual structure and function (Fox *et al.*, 2008); reaction time, balance, neurotransmission, long term potentiation (LTP) (Lasley and Gilbert, 1999; Lasley and Gilbert, 2002); and other CNS endpoints. Adverse effects on some of these CNS endpoints have been produced by blood lead concentrations in animals as low as $12 \mu g/dL$ (Fox *et al.*, 2008).

Adverse neurobehavioural effects of lead exposure have been demonstrated in multiple animal species, including:

- Impaired learning and motor coordination in herring gull (*Larus argentatus*) chicks (Burger and Gochfeld, 1997; Burger and Gochfeld, 2005)
- Impaired learning in prenataly lead exposed lambs (Carson *et al.*, 1974)
- Altered spatial exploration in offspring of paternally lead exposed rabbits (Nelson *et al.*, 1997)
- Decreased running speed and maze learning in short-tailed opossum (*Monodelphis domestica*) (Punzo and Farmer, 2004)
- Increased aggressive behaviour in golden hamster (*Mesocricetus auratus*) (Delville, 1999)
- Impaired social development, including increased male aggression, in offspring of maternally exposed mice (Donald *et al.*, 1987)

The most extensive and informative literature, however, is from experiments with non-human primates and rats.

There are at least 17 published reports of adverse neurobehavioural effects of lead in experiments with non-human primates. Not every endpoint examined, nor every exposure dose, nor every exposure period was associated with adverse outcomes. However, only one publication that reported primarily negative results was located at the time of this report (Laughlin *et al.*, 1999). The preponderance of evidence supports a causal relationship between lead exposure and neurobehavioural impairments in experiments with non-human primates. Adverse effects, however, are dependent on the difficulty of the task, functional domain tested, lead exposure period and dose. Adverse effects have repeatedly been reported in non-human primates with a history of lead exposure that produced mean blood lead concentrations in monkeys as low as 11-15 μ g/dL. Most studies were done using oral exposure to lead acetate in drinking water, gelatine capsules, or milk. No threshold for neurodevelopment effects of lead exposure in non-human primates has been clearly established.

There are at least 28 published reports of adverse neurobehavioural effects of lead in experiments with laboratory rats. Not every endpoint examined, nor every exposure dose, nor every exposure period was associated with adverse outcomes. However, only six publications that reported primarily negative results were located. The preponderance of evidence supports a causal relationship between lead exposure and neurobehavioural impairments in experiments with laboratory rats. Adverse effects, however, appear to depend on the difficulty of the task, domain tested, lead exposure period and dose. Adverse effects have repeatedly been reported in laboratory rats with a history of lead exposure that produced blood lead as low as 15-20 µg/dL. A single study reports adverse effects associated with blood lead concentrations as low as approximately 10 µg/dL (Cory-Slechta and Thompson, 1979). Most rat studies have used oral exposure to lead acetate in drinking water. No threshold for neurodevelopment effects of lead in laboratory rats has been clearly established.

Chronic oral exposure to lead in laboratory animals results in impaired performance on behavioural tests of cognitive function. These effects have been demonstrated in multiple species including at least two species of non-human primate. Statistically significant impairment on behavioural tests of cognitive function have been reported in animals with average concurrent blood lead concentrations as low as approximately10 µg/dL. Neurobehavioural effects in animals have been shown to persist after cessation of lead exposure and blood lead concentrations have returned to normal. No threshold for lead induced behavioural deficits in laboratory animals has been established. The collective *in vivo* evidence also illustrates the potential complexity of the lead-CNS exposure-response relationships. Under controlled conditions, these experiments revealed significant inter-individual variability in response. The magnitude and direction of response is also dependent on the magnitude, duration, and timing of exposure as well as the life stage at which the outcome is assessed. Neurobehavioural effects also have a longitudinal nature to their pattern of response (i.e. the magnitude or direction of effects can change with time since exposure). These observed complexities under controlled experimental conditions help explain some of the challenges and inconsistencies in detecting a lead-IQ effect in observational studies.

Mechanisms

Lead has been shown to interact with all cell types in the CNS. There is, to varying degrees, evidence to support a wide range of mechanisms that could result in impaired cellular functioning and survival leading to impaired CNS function. These include lead induced apoptosis; decreased cellular respiration; dysfunction in neurotransmitter synthesis, storage, release, and reuptake; oxidative stress; Ca²⁺ and Zn²⁺ mimicry and associated disruption in homeostatisis and protein function; impaired synaptic plasticity; disturbances in glial cell functioning; dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis and related interaction with the mesocorticolimbic dopamine system; and alteration of the epigenome. Cellular effects have been demonstrated *in vitro* at Pb²⁺ exposure concentrations as low as the picomolar range. The equivalent *in vivo* whole blood lead concentrations are uncertain, but are likely within the range of current environmental lead exposures in

Canada and possibly 100-fold lower². Several cellular and biochemical effects of lead exposure appear to have complex exposure-response relationships that may be biphasic or dependent on duration, magnitude, timing of exposure (for *in vivo* experiments), sex, brain region, or co-exposure to other stressors. In summary, there are several well supported mechanistic explanations for the neurotoxicity of lead and this line of evidence corroborates the conclusions of epidemiological studies and *in vivo* experiments.

Critical Biomarker-Endpoint Relationships for Developmental Neurotoxicity

The weight of evidence from all lines of inquiry (epidemiological, *in vivo* animal studies and *in vitro* experiments) supports an association between lead exposure and developmental neurotoxicity. Further, the epidemiological data base is sufficient to provide a quantitative estimate of the expected children's blood lead concentration-IQ relationship.

The strongest evidence of the developmental neurotoxicity of lead is derived from the studies of children's blood lead concentrations and measures of psychometric intelligence (IQ). Therefore, it is recommended that a TRV be developed based on the evidence of an association between early life blood lead and IQ decrements in school-aged children. Because there is insufficient evidence to conclude that the developing fetus is at least as sensitive as the child, this TRV should also apply to protection of the fetus and, therefore, by extension, to women of childbearing age.

² A whole blood Pb concentration in the general systemic circulation of 10 μ g/dL may be associated with a free Pb²⁺ concentration within the CNS of as low as 0.006 μ M (0.48 μ M x 0.00125) or 6 nM. The *in vitro* cellular effects of Pb²⁺ have been demonstrated at Pb²⁺ concentrations three orders of magnitude lower than this - in the low picomolar range.

While the available results are suggestive, overall there is a relative paucity of epidemiological evidence on the relationship between bone lead and IQ or other reliable measures of neurological development or functioning. Therefore, no bone lead based TRV can be reliably derived for early life lead exposure and developmental neurotoxicity.

Cardiovascular Toxicity

There are multiple lines of evidence, including human epidemiological studies, *in vivo* animal assays, and *in vitro* experiments, that support the hypothesis that chronic lead exposure can result in adverse cardiovascular effects, including increased blood pressure, in humans.

Lead exposure is associated with adverse effects on various cardiovascular endpoints including blood pressure, hypertension, inotropic and chronotropic cardiotoxicity, peripheral arterial disease (Navas-Acien *et al.* 2004), and coronary and cerebrovascular morbidity and mortality (Lustberg *et al.* 2002; Menke *et al.* 2006; Schober *et al.* 2006; Navas-Acien *et al.* 2007; Navas-Acien *et al.* 2008). The endpoint, however, that has been most studied and for which there is the greatest weight of evidence of a causal relationship is lead induced increases in blood pressure, particularly systolic blood pressure (SBP). Secondly, the other cardiovascular effects associated with lead exposure may be secondary effects of elevated blood pressure, although there is also evidence of independent and directly cardiotoxic effects of lead.

Epidemiological Evidence of Cardiovascular Toxicity of Lead

There have been a large number of epidemiological studies of the association between human lead exposure and blood pressure or risk of hypertension. Many, but not all, epidemiological studies report a significant, but weak association. A significant positive association has been reported among diverse study populations with varied ethnicity and socioeconomic status. A significant positive association remained in many studies after adjusting for a variety of potential confounding variables. Several meta-analyses report a weak, significantly positive association and three of six longitudinal studies published since 1980 report a significant association between blood lead and SBP.

The modest effect size and the inconsistency of the epidemiological results can be attributed, in part, to measurement error in both lead exposure and blood pressure. The effect of random measurement error in these parameters is null biasing. In contrast to studies that have relied on blood lead as a measure of exposure, bone lead has more consistently been associated with increased blood pressure or risk of hypertension (Navas-Acien et al. 2008). The single study that measured blood pressure with a 24 hour ambulatory monitoring device reported no association with blood lead (Staessen et al. 1996). Another source of variability in outcome is the degree to which the many potentially confounding variables, such as age, body mass index, socioeconomic status, sodium intake, drinking and smoking have been measured and controlled for. Effect modifiers, such as ethnicity or menopausal status, may also add to the variability in the reported data. On balance, the reported null associations between human lead exposure and blood pressure or risk of hypertension can equally be interpreted as evidence of the limitations of existing epidemiological investigations to detect an association as they can be interpreted as evidence of an absence of an association.

The epidemiological evidence of an association between tibia lead and elevated blood pressure is also inconsistent but suggestive. A significant positive association, after adjusting for covariates, has been found between tibia lead and SBP in three of the seven cohorts where this relationship has been studied.

There is insufficient evidence to derive a TRV on the basis of the potential cardiovascular effects of lead in children. Only one of five studies published before the mid 1990's on the relationship between children's (< 10 years of age) blood lead and blood pressure reported a significant positive association. Two of the three recent prospective epidemiological studies (Factor-Litvak et al. 1996; Chen et al. 2006) report no significant effect of blood lead and blood pressure. The recently reported results from the Oswego Children's Study (Gump et al. 2005) suggest that early life lead exposure can result in cardiovascular effects that may initially have little net effect on blood pressure, but which may ultimately lead to hypertension or other cardiovascular morbidity and mortality. These results, however, have not been replicated. Gump et al. (2005) also reported an association between maternal cord blood lead and SBP in children at 9 years old. No published studies on the relationship between bone lead and blood pressure in children were located for this report. Considered collectively, the current epidemiological evidence is insufficient to support an association between lead exposure and increased blood pressure in children.

Experimental Evidence of Cardiovascular Toxicity of Lead

Both chronic and subchronic oral exposure to lead in laboratory animals results in elevated blood pressure. The results of more recent animal studies have consistently shown an effect at relatively low levels of lead exposure. Chronic experimental exposure of mature male laboratory rats to 100 ppm lead (as lead acetate) in drinking water has repeatedly resulted in a significant increase in blood pressure (BP) associated with blood lead concentrations as low as 2.4 \pm 0.6 μ g/dL (Attri *et al.* 2003). Significant increases in BP have been observed at this exposure level following as little as 8 weeks of exposure (Ding *et al.* 1998).

There have been at least seven published laboratory studies reporting a statistically significant increase in BP in rats sub-chronically or chronically exposed to lead in drinking water with blood lead concentrations < 20 μ g/dL (Khalil-Manesh *et al.* 1994; Gonick *et al.* 1997; Vaziri *et al.* 1997; Ding *et al.* 1998; Vaziri *et al.* 1999a; Vaziri *et al.* 1999b; Ding *et al.* 2001; Attri *et al.* 2003); of these, four reported a statistically significant increase in BP with blood lead concentrations < 10 μ g/dL (Khalil-Manesh *et al.* 1994; Ding *et al.* 1998; Vaziri *et al.* 1999a; Vaziri *et al.* 1999b; Ding *et al.* 1998; Vaziri *et al.* 2003); of these, four reported a statistically significant increase in BP with blood lead concentrations < 10 μ g/dL (Khalil-Manesh *et al.* 1994; Ding *et al.* 1998; Vaziri *et al.* 1999a; Vaziri *et al.* 1999b). BP effects at these exposure levels have been observed in two different rat strains, but all of these studies were exclusively on male animals. None of the recent, low dose studies have identified a threshold for the hypertensive effects of lead.

The collective evidence from epidemiological studies and *in vivo* and *in vitro* experimental studies clearly indicate that there are several mechanisms by which chronic lead exposure could cause elevated blood pressure and related cardiovascular disease. The literature contains evidence that lead depresses nitric oxide (NO) and impairs NO signalling, induces vascular inflammation, increases adrenergic sensitivity, increases endothelins, interferes with the functioning of the rennin-angiotension-aldosterone and kininergic systems, upsets the balance of vasodilator and vasoconstrictor prostaglandins, induces calcium dependent and calcium independent contractility of vascular smooth muscle cells, stimulates proliferation and phenotypic transformation of vascular smooth muscle cells, inhibits proliferation of endothelial cells and impairs angiogenesis and endothelial cell repair, and inhibits sodium-potassium adenosine triphosphatase. Much of the evidence of these effects is derived from chronic studies at environmentally relevant lead

exposures. Lead exposure may also induce hypertension as a secondary effect of cardiotoxicity or nephrotoxicity. However, it is important to note that the hypertensive effects of lead have been repeatedly demonstrated independent of adverse cardiac or nephrotoxic effects.

Lead-induced hypertension may result from increased cardiac inotropism (increased stroke volume and cardiac output) and increased peripheral resistance. There is evidence that lead can affect hypertension via both of these pathways but the direct effects on peripheral resistance appear more sensitive and pronounced.

Critical Biomarker-Endpoint Relationships for the Cardiovascular Toxicity of Lead

The cardiovascular biomarker-endpoint relationship that has been most studied and for which there is the greatest weight of evidence of a causal relationship is increased blood pressure, particularly systolic blood pressure (SBP), and blood lead concentrations in adults. SBP was selected as the critical endpoint because it has been reported more frequently than diastolic blood pressure (DBP) and, where they have been both reported, lead has a stronger effect on SBP. SBP may also be a more important risk factor for cardiovascular morbidity and mortality than DBP.

Blood lead and hypertension risks are the endpoints that have been most commonly related to bone lead concentrations. In an earlier draft of this report a TRV for lead was developed on the basis of the relationship between tibia lead and SBP. While the literature allows for the development of a quantitative estimate of the relationship between tibia lead and SBP, there is greater uncertainty in this relationship than that of blood lead and SBP. Also, the slope of the tibia lead-SBP relationship did not appear to be consistent with that developed for blood lead-SBP. Reconciling these

differences was difficult because of the uncertainty in the relationship between cumulative blood lead index (CBLI) and tibia lead concentrations, particularly at relatively low environmental lead exposures. Therefore, it was decided that a TRV should not be derived on the basis of tibia lead and SBP in the final version of this report.

At the time of this report, no published studies on the relationship between bone lead and BP or hypertension in children could be located. Therefore, the scope of the review of epidemiological evidence of lead exposure and effects on BP or hypertension in children was limited to studies with blood lead as a biomarker. There is some evidence of vascular toxicity of lead in children. However, as a whole, this endpoint is considerably less studied in children and the weight of evidence and the quantification of a dose-response relationship are not strong enough to support the derivation of a TRV explicitly on the basis of lead's vascular toxic effects in children. However, the TRV derived for developmental neurotoxicity of lead will also infer an unquantified level of protection against potential vascular and other toxic effects of lead in children.

Estimated Blood Lead Concentration-Response Slopes

It is recommended that quantitative estimates of the blood lead concentrationresponse relationships for IQ in children and systolic blood pressure (SBP) in adults be used, with an emphasis on quantifying blood lead concentration-response relationships over the range of current blood lead concentrations in Canada. Estimates of the blood lead concentration-response relationships are provided so that risk assessors can estimate the change in population health outcomes associated with incremental changes in environmental lead exposures. Estimates of the slopes of the blood lead concentration-response relationships can be made with a relatively high degree of scientific certainty because blood lead concentrationresponse data are available from epidemiological studies of blood lead concentrations that are within, or very close to within, the range of current blood lead concentrations in Canada.

Current Blood Lead Concentrations in Canada

Preliminary results from the nationally representative Canadian Health Measures Survey (CHMS) report that the 25th and 95th percentiles of blood lead concentrations among Canadians 20 to 79 years old in 2007 and 2008 were approximately 1 and 4 μ g/dL, respectively. The 25th and 95th percentiles of blood lead concentrations in children six to 19 years of age are 0.6 and 1.6 μ g/dL, respectively.

There are no nationally representative data available for Canadian children less than six years of age. However, by several lines of reasoning it is estimated that the 25^{th} and 95^{th} percentiles of blood lead concentrations among Canadian children less than six years old are 1 and 4 to 6 µg/dL, respectively.

The Shape and Extent of the Blood Lead-IQ Relationship

The collective evidence suggests that the inverse association between early-life lead exposure and developmental neurotoxicity extends to the lower range of blood lead concentrations reported in the literature. There is relatively little evidence of a threshold above the lower range of blood lead concentrations studied and what evidence exists is limited to the results of categorical analyses from cross-sectional studies. This evidence is given limited weight due to the methodological limitations of these approaches. Nonetheless, it is important to note that one of the negative studies, that of Surkan *et al.* (2007), appears to have the largest number of study

subjects with blood lead concentrations less than 5 μ g/dL and also shows no inverse relationship between blood lead and IQ at blood lead concentrations less than 5 μ g/dL. While this study raises a degree of uncertainty, the preponderance of evidence support an inverse association, with no clear threshold, between blood lead concentrations and children's scores on tests of psychometric intelligence. Non-linear modeling from multiple study cohorts demonstrates that the concurrent blood lead concentration-IQ relationship extends as low as 1 to 2 μ g/dL and a single publication shows this relationship extending to as low as 0.5 μ g/dL. Categorical analysis provides supporting evidence and a single study using this approach demonstrates a significant adverse effect on tests of neonatal intelligence as maternal blood lead concentrations rise from 0.28 to 1.18 μ g/dL. The preponderance of evidence also demonstrates that the slope of the blood lead-IQ relationship becomes steeper at blood lead concentrations less than about 7.5 to 10 μ g/dL.

Critical Study for Defining the Slope of the Blood Lead-IQ Relationship

An international pooled analysis of seven longitudinal studies by Lanphear *et al.* (2005) was selected as the critical study that best represents the expected blood lead concentration-IQ relationship among environmentally exposed Canadian children.

Lanphear *et al.* (2005) reported on a pooled analysis of data from seven of the eight longitudinal prospective studies that were initiated prior to 1995 and followed subjects until at least five years old. The analysis involved 1,333 children with complete data on requisite covariates. The participating studies included Boston, MA; Cincinnati, OH; Cleveland, OH; Rochester, NY; Mexico City; Port Pirie, Australia; and Kosovo, Yugoslavia. Data from the Sydney, Australia cohort was not included in the pooled analysis. Full scale IQ was the outcome assessed. Subject's IQ was assessed at primary school age (mean age of assessment 6.9 years; range 4.8-10 years; five of the seven studies assessed IQ at six or seven years old) with an age and language appropriate version of the Wechsler Intelligence Scales for Children (WISC-R, WISC-III, WPPSI, or WISC-Spanish).

Children's blood lead was sampled by venous or capillary sample, depending on the protocols of the individual participating studies. Four blood lead indices were used in the analysis: (1) concurrent; (2) maximum; (3) lifetime average; and (4) early childhood, defined as the six month to two year mean blood lead. Data on cord blood lead was also available for 696 subjects. The pooled median concurrent, maximum, lifetime average and early childhood blood lead was 9.7 μ g/dL, 18.0 μ g/dL, 12.4 μ g/dL, and 12.7 μ g/dL, respectively. One hundred and three (8%) of subjects had a maximum blood lead < 7.5 μ g/dL.

Data on the following covariates were included in the pooled analysis: maternal IQ, education, marital status and prenatal alcohol and tobacco use; HOME Inventory score; and subject sex, birth order, and birth weight. The influence of ethnicity was investigated for the sub-set of US data. Potentially important covariates that were not included in the pooled analysis include SES and nutritional status.

Multiple fixed effect regression modeling was used to investigate the associations between blood lead indices and IQ. In a comparative analysis of the respective coefficients of determination of the linear regression models, concurrent blood lead was identified as the blood lead index that explained the greatest variance in IQ.
Quadratic and cubic terms were added to the adjusted linear regression models to test for linearity. Both the quadric and cubic terms were statistically significant and a restricted cubic spline model was used to illustrate the shape of the blood leadresponse relationship. A log-linear model was identified as the parametric function that most closely matched the shape of the blood lead-response relationship indicated by the restricted cubic spline model.

After adjusting for covariates, significant inverse associations were reported between all four blood lead indices and full scale IQ. A significant inverse association was also reported for concurrent blood lead and performance and verbal IQ. Ethnicity did not affect the concurrent blood lead-IQ relationship for the subset of subjects from the US studies where data on this variable was available. The inverse relationship between cord lead and IQ was not statistically significant after adjusting for covariates.

Linear regression models were also constructed for various maximum blood lead cut-points that had been identified *a priori*. The adjusted linear regression coefficient between concurrent blood lead and IQ for subjects with a maximum blood lead < 7 μ g/dL was significantly less than (i.e. steeper negative slope) that for subjects with a maximum blood lead \geq 7 μ g/dL. The magnitude of difference in the slopes of the blood lead-response relationship was about 20-fold. The difference in blood lead-response for subjects with a maximum blood lead of < 10 μ g/dL was not significantly different from those subjects with a maximum blood lead of \geq 10 μ g/dL. This analysis provides additional strength to the evidence that the blood lead-IQ becomes shallower at blood lead concentrations greater than about 7-10 μ g/dL.

Sensitivity analysis demonstrated that the results of the pooled analysis were not driven by any single particular study cohort or the use of a fixed effects model rather than random effects model.

Lanphear et al. (2005) was selected as the critical study for the following reasons:

- Of the existing longitudinal study publications, Lanphear *et al.* (2005) has, by far, the highest number of and greatest diversity of subjects. The analysis included seven of the eight longitudinal data sets on children's blood lead and IQ available at the time of the analysis. The demographic characteristics of the individual study cohorts included in the pooled analysis span a wide range of ethnic, social, and economic variability and, of the available studies, best reflects the ethnic and social diversity of the Canadian population.
- The study examined the potential influence of 10 covariates, and data on these covariates were available for a high proportion of subjects (84%). One potentially important covariate that was not included in the Lanphear *et al.* (2005) pooled analysis is a direct measure of household wealth. However, the pooled analysis did include an analysis of maternal age at delivery, maternal IQ, maternal education at delivery, and ethnicity, all of which are correlated with SES.
- The data were from prospective studies with serial blood measures. It was demonstrated that higher, unmeasured blood lead concentrations from early life exposures were not driving the observed results.
- The authors of the paper provided evidence to justify their selection of concurrent blood lead as the primary lead exposure index.
- An independent published analysis of the pooled data verified that the functional form of the model used in the critical study provided a better fit to the study data than alternate models.

- Evidence was provided in the original publication and in subsequent correspondence with the study authors that the selected functional forms of the models provided better fits to the underlying study data than alternate forms.
- Model stability and sensitivity was assessed, and evidence was provided to demonstrate that the results of the pooled analysis were not overly dependent on the data from any single study, nor were the results significantly affected by exclusion of potential outlier data.

Concurrent blood lead (i.e.: a blood sample was collected at the same time that the subject's IQ was assessed) explained the greatest variance in IQ among the subjects of the pooled analysis. Therefore, concurrent blood lead was the exposure index selected to quantify the recommended blood lead-IQ exposure response relationship. While the regression coefficients for other blood lead indices differed by up to 25%, they are within the overall range of uncertainty in the slope of the blood lead concentration-IQ relationship. The slope for concurrent blood lead also provides a reasonable estimate of the slope for the other blood lead indices (early childhood, peak, and lifetime average).

Uncertainty in the Slope of the Blood Lead-IQ Relationship

There are multiple sources of variability and uncertainty in the estimated slope of the blood lead concentration-IQ relationship. These include:

- Variability in the slope of the relationship among the published studies
- Uncertainty about what model best describes the underlying relationship
- Uncertainty in the parameters of the model fit to the data of the critical study

- Uncertainty about the influence of bias and confounding
- Inter-individual variability in susceptibility and the resulting variance in the slope of the blood lead concentration-response relationship
- The blood lead index used to estimate exposure
- The relative timing, absolute age, and form of the IQ test

Selection of the Critical Study

The use of a pooled analysis as a critical study somewhat attenuates the potential influence of inter-study variability in the reported blood lead concentration-response relationship. However, some important quantitative differences between the slope of the relationship based on the critical study and published results from other analyses were noted: The recommended slope based on the log-linear model of Lanphear *et al.* (2005) is less steep than the two other available analyses where a log-linear functional form of the relationship was demonstrated to be statistically superior to a linear model. The other two log-linear models are not directly comparable, however, because of differences in measures of lead exposure and psychometric intelligence. The recommended slope based on the log-linear model of Lanphear *et al.* (2005) is in close agreement with the adjusted regression coefficients of most linear models based on blood lead concentrations less than 10 μ g/dL. There are, however, a few exceptions.

Uncertainty in the Functional Form of the Relationship

While there is some uncertainty in the choice of the functional form of the model that best represents the blood lead-IQ relationship, the preponderance of evidence indicates that the shape of the relationship, over the range that it has been studied,

is supralinear with a steeper slope and little evidence of a threshold at relatively low blood lead concentrations.

Bias and Confounding

The observed relationship is unlikely to be entirely attributable to confounding, as there is strong evidence of biological plausibility. This association has been observed in multiple cohorts of divergent ethnic, social and economic demographics, and the association has been repeatedly observed in longitudinal cohort studies. While the magnitude of the slope of the recommended relationship between mean population IQ and concurrent blood lead in children is undoubtedly influenced to some unknown degree by confounding, it is also likely attenuated by over-control. No evidence that the observed association from the critical study was unreasonably affected by selection, measurement or other bias was identified.

Variance in Susceptibility

There is some evidence of the potential modifying effects of environmental stress and genetic polymorphisms on the relationship between lead exposure and neurotoxicity. However, the blood lead concentration-IQ relationship for this endpoint was derived from a large international pooled analysis of study populations of diverse ethnicity and socioeconomic status. The variability in susceptibility among the cohorts included in the Lanphear *et al.* (2005) pooled analysis was judged to be sufficiently representative of the variability in susceptibility that might be expected across the Canadian population.

Accounting for Variability and Uncertainty

The 95th percent confidence intervals on the adjusted log-linear regression coefficient from the critical study are recommended to partially quantify the potential influence of these sources of variability and uncertainty. However, the confidence intervals are derived only based on the uncertainty in the value of the model parameter (the regression coefficient). They do not quantitatively account for all other sources of uncertainty and variability in the overall estimate of the blood lead concentration-response relationship. The upper and lower estimates of the blood lead lead concentration-response relationship represent plausible, but less likely, values for the slope of the relationship. They do not bound the entire range of reported slopes of the blood lead concentration-response relation-response relationship, but there are relatively few data supporting estimates of the blood lead concentration-response relationship outside of this range.

Recommended Slope of the Blood Lead-IQ Relationship

The recommended slope of the relationship between population mean IQ and concurrent blood lead in children is based on the adjusted regression coefficient of the log-linear model from the critical study by Lanphear *et al.* (2005). The covariate adjusted log-linear slope of the relationship between IQ and concurrent blood lead for the entire pooled data is -2.70 IQ points per natural log unit (μ g/dL) increase in concurrent blood lead. The reported 95th percent confidence intervals on the log-linear slope are -1.66 to -3.74 IQ points per natural log unit (μ g/dL) increase in concurrent blood lead. The minimum and 95th percentile concurrent blood lead from the pooled analysis were 0.5 and 33.2 μ g/dL, respectively. While the 95th percent confidence intervals in the slopes from the critical study are used to derive quantitative estimates of the uncertainty and variability in the blood lead concentration-response relationship, it is recommended that these slopes be defined

more generally as upper and lower estimates of the potential variability and uncertainty in the slope to avoid misunderstandings about the potential precision of the estimates. While the minimum concurrent blood lead concentration from the critical study was 0.5 μ g/dL, the observed association has only been independently replicated in studies with blood lead concentrations as low as 1 μ g/dL. It is, therefore recommended, that this slope of the critical study only be considered validated down to concurrent blood lead concentrations of 1 μ g/dL.

Using the recommended slope of the blood lead-IQ relationship from the critical study, a change in concurrent blood lead in children from 1.0 μ g/dL to 4.0 μ g/dL, which is estimated to be approximately equivalent to the 25th to 95th percentile of blood lead concentrations in Canadian children 5 to 10 years old, is associated with a change in mean IQ among those children of approximately -2.3 to -5.2 IQ points, with a best estimate of -3.7 IQ points. The potential range of IQ loss may be larger or smaller because the estimates provided do not account for all potential sources of uncertainty and variability in the blood lead concentration-IQ relationship.

It must be stressed that this population IQ loss should not be confused with individual IQ loss. This modeled relationship cannot be interpreted to predict that the IQ of an individual child would decline by 3.7 IQ points if the child's blood lead concentration increased from 1 to 4 ug/dL. Instead, the average IQ of a population of children with a blood lead concentration of 4 ug/dL is expected to be 3.7 IQ points lower than a comparable population of children with a blood lead concentration of children with a blood lead concentration of 2 ug/dL is expected to be 3.7 IQ points lower than a comparable population of children with a blood lead concentration of 1 ug/dL.

Adult Blood Lead and SBP

Critical Study for Defining the Slope of the Blood Lead-SBP Relationship

Similar to the epidemiological studies of children's blood lead and IQ, there is a great deal of inter-study variability in the reported blood lead concentration-response relationships for adult blood lead and SBP. Unlike the blood lead-IQ relationship, there is no pooled analysis or suitable meta-analysis that provides a combined estimate of the blood lead concentration-response relationship for blood lead and SBP. At the time of this report, no published pooled analysis of this relationship had been located in the literature. There are several published meta-analyses, but it was decided that there was too much heterogeneity among the individual studies included in the meta-analyses for the combined estimate to provide a reasonable estimate of the expected blood lead concentration-response relationship among environmentally exposed Canadians.

Prospective environmental cohort studies were given preference as critical studies. Six longitudinal studies of the association between blood lead and SBP published since 1980 were identified (Weiss *et al.*, 1986; Neri *et al.*, 1988; Moller and Kristensen, 1992; Staessen *et al.*, 1996; Glenn *et al.*, 2003; Glenn *et al.*, 2006). Three of six longitudinal studies that included SBP as an endpoint reported a significant positive association with blood lead after adjusting for covariates, but none of these were environmental cohort studies. Therefore, it was not possible to select a longitudinal study of an environmental cohort as the critical study for this endpoint.

The study conducted by Glenn *et al.* (2003) was of a formerly occupationally exposed cohort, although the blood and bone lead concentrations in this cohort were comparable to environmentally exposed subjects at the time of the study (mean blood lead at baseline was 4.6 μ g/dL with a standard deviation of 2.6 μ g/dL; mean tibia lead at year three of the study was 14.7 μ g/g with a standard deviation of 9.4 μ g/g). The study population was occupationally exposed for 18 years, on

average, prior to the study. The association between baseline blood lead and annual change in SBP was stronger for the subset of subjects who had lower past peak tibia lead concentrations than for the whole cohort combined. This suggests that it is not merely higher historical exposures that are driving the observed relationship between blood lead and SBP. Therefore, the longitudinal study by Glenn *et al.* (2003) best met the selection criteria for a critical study to estimate the relationship between adult blood lead and SBP among environmentally exposed Canadians.

The subjects in the study by Glenn *et al.* (2003), however, are not adequately representative of the diversity of the Canadian population. All of the predominantly Caucasian subjects in the study of Glenn *et al.* (2003) were relatively healthy and male. The mean (standard deviation) age of the subjects at baseline (1994-1996) was 55.8 (7.4) and they had a SBP of 129.3 (13.9) mmHg. In comparison, the mean (standard deviation) blood pressure in male Canadians aged 55 to 64 during the period 1986-92 was 137.3 (17.0) mmHg (Canadian Heart Health Database, 1992).

There is evidence to suggest that ethnicity, sex, stress, nutritional status, coexposure to other xenobiotics, and genetics may modify the concentration-response relationship between blood lead and SBP. The reported adjusted slopes of the relationship between blood lead concentration and SBP vary by over an order of magnitude. In the context of the evidence of potential inter-individual variability in the blood lead concentration-response relationship between lead and SBP and the limited representativeness of the study cohort of Glenn *et al.* (2003), it was necessary to include an additional critical study to characterize the upper range of the reported variance in observed blood lead concentration-response relationships for blood lead and SBP. Because no other longitudinal studies reporting positive results were suitably representative of environmentally exposed Canadians (all were from currently occupationally exposed cohorts), cross-sectional studies of the relationship between blood lead and SBP were used instead.

It is recommended that the slope for African-American women from the study of Vupputuri *et al.* (2003) be used for an upper estimate of the potential variance in the slope of the blood lead-SBP relationship. The adjusted regression coefficient for the relationship between blood lead and SBP among African-American female subjects of the Vupputuri *et al.* (2003) study represents the steepest slope of the blood lead-SBP relationship for a potentially susceptible sub-population *that has been identified as a susceptible sub-population in multiple studies.* Den Hond *et al.* (2002) also reported a stronger relationship between blood lead and SBP for African-American women, as compared to Caucasian males. In the absence of any other strongly differentiating criteria, the 95% upper confidence limit of the adjusted regression coefficient for the relationship between blood lead and SBP among African-American female subjects of Vupputuri *et al.* (2003) was selected as the upper limit estimate of the slope of the relationship between adult blood lead and SBP.

The critical studies of Glenn *et al.* (2003) and Vupputuri *et al.* (2003) and the associated slopes of the relationship between blood lead and SBP are described in more detail below.

Glenn *et al.* (2003) analyzed the longitudinal association between blood lead concentration and annual change in BP among 496 former and current male employees of a chemical-manufacturing facility in the Eastern USA. The facility historically produced tetramethyl and tetraethyl lead. The average age at baseline was 55.8 (SD \pm 7.4) years, the average time since occupational lead exposure was 17.7 \pm 11.6 years, and the average blood lead at baseline was 4.6 \pm 2.6 µg/dL, with a range of 1 to 20 µg/dL. The mean SBP was not reported by Glenn *et al.* (2003), but

in an earlier report on 543 subjects from the same cohort. Schwartz *et al.* (2000b) reported a mean (and standard deviation) SBP of 127.9 ±15.3 mmHg. Subjects were followed for an average of 2 (range 0.85-3.5) years and BP was assessed three or four times over this follow-up period. Sitting BP was measured using a random zero sphygmomanometer. BP was recorded as the average of three readings to the nearest 2 mmHg taken at five-minute intervals. Generalized estimating equations (GEE) were used to evaluate the relationship between baseline blood lead and annual change in BP, while controlling for other covariates. After adjusting for baseline age, body mass index, antihypertensive medications, smoking, education, technician and the time to each BP measurement, SBP increased at an average annual rate of 0.64 (95% CI: 0.14-1.14) mmHg for every standard deviation (2.6 μ g/dL) increase in baseline blood lead. This is equivalent to an adjusted linear dose-response slope of 0.25 mmHg per year per μ g/dL with 95% confidence intervals of 0.05 to 0.45 mmHg per year per μ g/dL Significant positive associations were also reported between tibia lead and SBP and peak tibia lead and SBP.

To make the longitudinal slope reported by Glenn *et al.* (2003) comparable to the cross-sectional relationship reported by Vupputuri *et al.* (2003), it was assumed that the slope of the relationship was absolute and it was simplified to 0.25 (0.05 to 0.45) mmHg per μ g/dL. This was viewed as a reasonable simplifying assumption since an earlier cross-sectional analysis of 543 subjects from the same cohort reported an adjusted linear regression coefficient (and SE) of 0.504 (0.249) mmHg per μ g/dL (Schwartz *et al.*, 2000b) (i.e., the longitudinal slope was within the 95% confidence intervals of the slope of the cross-sectional relationship).

It is recommended that the slope of the adjusted regression model from the critical study of Glenn *et al.* (2003) (0.25 mmHg per μ g/dL) be used to represent the best estimate slope of the relationship between adult blood lead and SBP among

Caucasian males. Furthermore, it is recommended that the lower 95^{th} percent confidence interval on the slope of the adjusted regression model from the critical study of Glenn *et al.* (2003) (0.05 mmHg per µg/dL) be used to provide a lower estimate of the slope of the relationship between adult blood lead and SBP among Caucasian males and other relatively less susceptible sub-populations.

Vupputuri et al. (2003) reported on the cross-sectional relationship between blood lead and SBP among 14,952 adult (>18 yrs) participants of the Third National Health and Nutrition Examination Survey (1988-94) (NHANES-III). Triplicate blood pressure measurements were made using a mercury sphygmomanometer during a home visit and at a mobile clinic. Subject blood pressures were calculated as the average of available measurements. Data were collected on the following potential covariates: age, education, body mass index, alcohol consumption, physical activity, dietary intake of sodium and potassium, and total dietary energy intake (as measured by 24hour dietary recall). No measure of poverty, an important potential confounder, was included. Subjects taking medication for hypertension were excluded from the study and analyses were weighted to account for the NHANES III sampling strategy. Multivariate-adjusted linear regression models were used to examine the crosssectional association between blood lead and BP, stratified by race and ethnicity. After adjusting for covariates, blood lead was significantly associated with higher SBP and DBP among African-American men (n=2,104) and women (n=2,300), but not Caucasians of either sex. The mean blood lead among the African-American women was 3.4 µg/dL with a standard error of 0.1 µg/dL. The minimum blood lead among subjects was 1 µg/dL (Vupputuri, 2009, pers. com.). The mean (SE) age of the African-American women was 42 (±0.4) years and their mean SBP was 122.4 (±0.6) mmHg. Vupputuri et al. (2003) do not report a regression coefficient and SE from their analysis, but instead report that a one standard deviation increase in blood lead (3.3 µg/dL) was associated with an adjusted increase (95th percent confidence

intervals) in SBP of 1.55 (0.47 to 2.64) mmHg. The calculated equivalent β_1 and 95th percent confidence intervals are: 0.47 (0.14 to 0.80) mmHg per μ g/dL. The regression model was adjusted for age, education, body mass index, use of alcohol, physical activity, sodium, potassium and total calories.

It is recommended that the slope of the adjusted regression model from the critical study of Vupputuri *et al.* (2003) (0.47 mmHg per μ g/dL) be used to represent the best estimate of the slope of the relationship between adult blood lead and SBP among potentially susceptible sub-populations. Potentially susceptible sub-populations include, but are not limited to, African-American women. Furthermore, it is recommended that the upper 95th percent confidence interval on the slope of the adjusted regression model from the critical study of Vupputuri *et al.* (2003) (0.80 mmHg per μ g/dL) be used to provide an upper estimate of the slope of the relationship between adult blood lead and SBP among susceptible sub-populations.

Uncertainty in the Slope of the Blood Lead-SBP Relationship

There are multiple sources of variability and uncertainty in the estimate of the slope of the blood lead concentration-SBP relationship. These include:

- Inter-individual variability in susceptibility and the resulting variance in the slope of the blood lead concentration-response relationship
- Uncertainty about what model best describes the underlying relationship
- Uncertainty in the parameters of the model fit to the data of the critical study
- Uncertainty about the influence of bias and confounding

Inter-Study Variance in the Reported Slope of Blood Lead Concentration-IQ Relationship There is evidence to suggest that ethnicity, sex, stress, nutritional status, coexposure to other xenobiotics, and genetics may modify the blood lead concentration-response relationship between lead and SBP. For example:

- Glenn *et al.* (2001) reported that the slope of the adjusted blood lead-SBP relationship for the 5% of subjects in their study cohort who were homozygous for the variant allele of the ATP1A2 gene was about twelve-fold higher than that for the balance of their cohort. The authors also reported that the variant allele was 1.8 times more prevalent among African-American subjects in their study cohort.
- Vupputuri *et al.* (2003) reported that the slope of the adjusted blood lead-SBP relationship for African-American women was about five-fold higher than that of Caucasian males. Den Hond *et al.* (2002) also reported a higher slope of the adjusted blood lead-SBP relationship for African-American women relative to Caucasian males.
- Peters *et al.* (2007) reported that the slope of the adjusted tibia lead-SBP relationship for subjects of the Normative Aging Study with high levels of self-reported stress was about 2.5 times higher than subjects with low stress.
- Vupputuri *et al.* (2003) also reported that the relationship between blood lead and SBP differed significantly between the sexes; in contrast, however, the meta-analysis of Nawrot *et al.* (2002) reported that the association between blood lead and SBP was similar between the sexes.
- Nash *et al.* (2003) reported that the relationship between blood lead and risk of hypertension was more pronounced in postmenopausal women than premenopausal women.

In the context of the evidence of potential inter-individual variability in the blood lead concentration-response relationship between lead and SBP and the limited

representativeness of the study cohort of Glenn *et al.* (2003), it was necessary to include an additional critical study to characterize the upper range of the reported variance in observed blood lead concentration-response relationships for blood lead and SBP. The adjusted regression coefficient for the relationship between blood lead and SBP among African-American female subjects of the Vupputuri *et al.* (2003) study represents the steepest slope of the blood lead-SBP relationship for a potentially susceptible sub-population that has been identified as a susceptible sub-population in multiple studies. The slope from Vupputuri *et al.* (2003) accounts for some, but not all, of the reported variance in slopes of the blood lead concentration-SBP relationship.

Uncertainty in the Extent and Shape of the Blood Lead-SBP Relationship

A literature search revealed few publications that explicitly tested the shape of the blood lead concentration-response relationship between blood lead and SBP or attempted to identify a threshold for the hypertensive effects of lead. In the absence of evidence to the contrary, it is recommended that the slope of the blood lead concentration-response relationship between blood lead and SBP be assumed to be linear. However, the certainty of the estimates of the slopes decreases over the lower range of the study data. The mean blood lead concentrations of the subjects of the critical studies were 4.6 μ g/dL (Glenn *et al.*, 2003) and 3.4 μ g/dL(Vupputuri *et al.*, 2003). Therefore, it is recommended that the estimates of the slope of the relationship between blood lead and SBP for blood lead concentrations below about 4 μ g/dL be accompanied by explicit statements that qualify the additional uncertainty inherent in the estimates of the slopes over this lower range; the slopes of the blood lead-SBP relationship have not been explicitly examined for non-linearity over the lower range of available data.

Bias and Confounding

The observed relationship is unlikely to be entirely attributable to confounding. Lead exposure causes hypertensive effects in experimental animals, there is strong evidence of biological plausibility, and the association between blood lead concentrations and SBP has been observed in multiple cohorts of divergent ethnic, social and economic demographics. While the magnitude of the slope of the recommended relationship between mean population SBP and blood lead in adults is undoubtedly influenced to some unknown degree by confounding, it is also likely attenuated by over-control. No evidence that the observed association from the critical studies were unreasonably affected by selection, measurement or other bias was identified.

Accounting for Variability and Uncertainty

The 95th percent confidence intervals on the adjusted log-linear regression coefficient from the critical study are recommended to partially quantify the potential influence of these sources of variability and uncertainty. However, the confidence intervals are derived only based on the uncertainty in the value of the model parameter (the regression coefficient). They do not quantitatively account for other sources of uncertainty and variability in the overall estimate of the blood lead concentration-response relationship. While the upper and lower estimates of the blood lead concentration-response relationship represent plausible, but less likely, values for the slope of the relationship, they do not bound the entire range of reported slopes. There are relatively few data supporting estimates of the blood lead concentration-response relationship outside of this range.

Recommended Slope of the Blood Lead-SBP Relationship

The longitudinal study of the relationship between baseline blood lead and change in SBP over time among former lead workers by Glenn et al. (2003) was selected as the critical study upon which to base a quantitative estimate of the slope of the relationship between blood lead and SBP. The slope and 95th percent confidence intervals of the adjusted linear regression coefficient reported by Glenn et al. (2003) is 0.25 (0.05 to 0.45) mmHg per year per µg/dL. To meet the requirements of risk assessors and to make the results comparable with other epidemiological studies, it was assumed that the relationship was cross-sectional (static) and simplified the slope to 0.25 (0.05 to 0.45) mmHg per µg/dL concurrent blood lead. It is recommended that the slope of the adjusted regression model from the critical study of Glenn et al. (2003) (0.25 mmHg per µg/dL) be used to represent the best estimate slope of the relationship between adult blood lead and SBP among Caucasian males. It is further recommended that the lower 95th percent confidence interval on the slope of the adjusted regression model from the critical study of Glenn et al. (2003) (0.05 mmHg per µg/dL) be used to provide a lower (less steep) estimate of the slope of the relationship between adult blood lead and SBP among Caucasian males and other potentially non-susceptible sub-populations.

Ethnicity, sex, stress, nutritional status, co-exposure to other xenobiotics, and genetics may modify the blood lead concentration-response relationship between lead and SBP. There is suggestive evidence that the slope of the blood lead concentration-response relationship may be steeper by up to a factor of about ten for susceptible sub-populations. In light of the observed variability in the slope of the relationship between blood lead and SBP, a slope is also recommended for susceptible sub-populations. The magnitude of this slope is based on the results of a cross-sectional linear regression analysis of the relationship between blood lead and SBP among African-American female participants of NHANES III (Vupputuri *et al.*, 2003). The slope is intended to be representative of all potentially susceptible sub-

populations and not exclusively African-American females. It was decided that the the recommended slope for susceptible sub-populations was based on a critical study of African-American females because evidence that identifies African-American females as a susceptible sub-population has been replicated in independent studies. It should be emphasized, however, that there are un-replicated data that suggest a steeper blood lead concentration-response relationship for some other susceptible sub-populations.

Vupputuri *et al.* (2003) do not report a regression coefficient and SE from their analysis, but instead report that a one standard deviation increase in blood lead (3.3 μ g/dL) was associated with an adjusted increase (95th percent confidence intervals) in SBP of 1.55 (0.47 to 2.64) mmHg. The calculated equivalent adjusted regression coefficient (and 95th percent confidence intervals) are: 0.47 (0.14 to 0.80) mmHg per μ g/dL. It is recommended that the slope of the adjusted regression model from the critical study of Vupputuri *et al.* (2003) (0.47 mmHg per μ g/dL) be used to represent the best estimate of the slope of the relationship between adult blood lead and SBP among potentially susceptible sub-populations. It is further recommended that the upper 95th percent confidence interval on the slope of the adjusted regression model from the critical study of Vupputuri *et al.* (2003) (0.80 mmHg per μ g/dL) be used to provide an upper estimate of the slope of the relationship between adult blood lead and SBP among susceptible sub-populations.

In addition to two slopes to represent the potential variability in blood lead concentration-response amongst sub-populations, it is also recommended that the upper and lower estimates of the blood lead concentration-response slopes to further characterize the potential variability and uncertainty in the estimate of the slope. The upper and lower estimates of the slopes are derived from the upper and lower 95th percent confidence intervals of the adjusted linear regression coefficients

from the critical studies. While these upper and lower estimates are based on, and to some degree account for, the uncertainty in the slope of the adjusted regression coefficient, they are based on the assumption that blood lead has been measured without error and do not explicitly account for additional sources of uncertainty (e.g., model uncertainty or the uncertain influence of bias and confounding). Therefore, it is not recommended that these upper and lower estimates of the slope of the relationship be gualified by inferring a degree of precision in the estimates (such as 95th percent confidence intervals). Rather, the upper and lower estimates of the blood lead concentration-response relationship represent plausible, but less likely, values for the slope of the relationship. They do not bound the entire range of reported slopes of the blood lead concentration-response relationship, but there are relatively few data supporting estimates of the blood lead concentration-response relationship outside of this range. The upper and lower estimates of the slope of the relationship between blood lead and SBP do not account for all sources of variability and uncertainty in the estimate of the slope of the relationship between blood lead and SBP.

It is emphasized that:

- The upper and lower estimates of the blood lead concentration-response relationship represent plausible, but less likely, values for the slope of the relationship
- The upper and lower estimates of the blood lead concentration-response relationship do not bound the entire range of reported slopes of the blood lead concentration-response relationship
- The slope of the relationship has not been tested for nonlinearity over the lower range of study data and estimates of the slope of the relationship are increasingly uncertain below blood lead concentrations of about 4 µg/dL

- Estimates of the blood lead-SBP relationship below 1 µg/dL are based on extrapolation of the relationship beyond the range of study data and the uncertainty in the estimate of the slope of the relationship further increases with increasing magnitude of extrapolation
- Alternate functional forms of the putative linear blood lead-SBP relationship have not been explored or tested
- The degree to which bias and confounding may have affected the quantitative estimates of the slope of the relationship between adult blood lead and SBP is uncertain

Based on the recommended slopes, a change in blood lead in adults from 1.0 µg/dL to 4.0 µg/dL, which is estimated to be approximately equivalent to the 25th to 95th percentile of blood lead concentrations in Canadian adults, is associated with an estimated increase in mean SBP among adults of approximately 0.2 to 2.4 mmHg, with a best estimate of an increase of 0.8 mmHg among Caucasian males and an increase of 1.4 mmHg among susceptible sub-populations. The potential range of increase in SBP may be larger or smaller because the estimates provided do not account for all potential sources of uncertainty and variability in the blood lead concentrationship.

Risk Specific Blood Lead Concentrations

The slopes of the blood lead-IQ and blood lead-SBP were extrapolated to the origin to calculate risk-specific blood lead concentrations.

The population risks associated with the risk-specific blood lead concentrations calculated in this report are:

- The average blood lead concentration in children that is associated with an average IQ loss of 1 point or less in 95% of the population. At this blood lead concentration, 5% of the population would have an average IQ loss of 1 point or greater.
- The average blood lead concentration in adults that is associated with an average increase in SBP of 1.3 mmHg or less in 95% of the population. At this blood lead concentration, 5% of the population would have an average increase in SBP of 1.3 mmHg or greater. An increase in SBP of 1.3 mmHg is approximately equivalent to a 1% increase in the average SBP among Canadian men and women 35 to 64 years old.

Methods

The general steps used to calculate risk-specific blood lead concentrations were:

- Define a benchmark level of risk (or response)
- Define the mathematical model of the blood lead concentration-response relationship
- Calculate the benchmark blood lead concentration associated with the benchmark risk
- Assume a population distribution for blood lead concentrations
- Calculate the geometric mean blood lead of distribution, where the 95th percentile of the distribution is equivalent to the benchmark blood lead concentration.

While some of the language and concepts from the benchmark dose (BMD) methods of deriving a TRV are used in this report, it is important to note that the traditionally applied BMD methods were not completely followed. Principally this is

because a dose-response model was not fit, *de novo*, to the critical study data. While this would have been desirable, the original study data was not obtainable and the critical studies all included published dose-response models. Additionally, in the case of IQ, the fit of the published dose-response model was well justified in both the critical study and in an independent published analysis of the same data.

A second departure from the commonly applied BMD method is that a benchmark response (BMR) relative to background or control was not calculated. This is because the epidemiological design of the critical studies did not measure this parameter. It was therefore assumed that at a blood lead concentration of 0 μ g/dL there will be no effect on IQ or SBP.

A third difference between the methods used in this report and the traditional BMD approach is that the lower confidence limit on the benchmark dose (BMDL) is normally used as a point of departure (POD) and the POD is divided by uncertainty factors to produce a "threshold" TRV. Because the objective of this report was to define the blood lead concentration associated with a specified level of risk, the confidence intervals (both upper and lower) on the BMD were used to help quantify the uncertainty in the estimate. The intervals employed are not referred to as precisely defined confidence intervals (e.g., 95th percent confidence intervals), because the statistical methods by which they were derived do not quantitatively account for all sources of variability and uncertainty in the estimate. The BMD in this report was not divided by any uncertainty factors because the objective was not to identify a POD for determining a threshold TRV, but simply to identify a specific level of response based on a blood lead concentration-response relationship. Finally, because the exposure metric in the critical studies is blood lead concentration, the benchmark exposures are referred to as benchmark concentrations (BMCs), rather than BMDs.

Benchmark Risks

The benchmark responses (BMRs) are defined as (1) an absolute increase in SBP of 1.3 mmHg (approximately equivalent to 1% of the average SBP among Canadian adults); and (2) an absolute decrement in IQ of 1 point (by definition equivalent to 1% of the national average IQ). It was also assumed that there is no lead effect at a blood lead concentration of 0 µg/dL (i.e., the intercepts of the blood lead concentration-response models were assumed to be zero). Therefore, the BMR for SBP was defined as 1.3 mmHg and the BMR for IQ was defined as -1 IQ point. By this method, the only parameter from the mathematical blood lead concentration-response relationship that is required for calculation of the benchmark concentration (BMC) is the slope of the model. In all cases, published adjusted regression coefficients from the critical studies were used as the model slopes for calculating BMCs.

Normal distribution theory was used to model the following dichotomous outcomes associated with a 1% increase in population mean SBP and a 1point decrement in population mean IQ: (1) the extra risk of mild mental retardation (MMR); (2) the extra risk of hypertension (Pre, Stage I and Stage II hypertension); and (3) the added risk of coronary heart disease (CHD) mortality.

The results of the normal distribution theory modeling were that:

- A 1 point decrement in population mean IQ is associated with an added risk of MMR of approximately 1 in 250 (385 in 100,000)
- The total sex and age-adjusted added risk of hypertension (Pre, Stage I and Stage II hypertension) among 35 to 74 year olds associated with a 1%

increase in population mean SBP is approximately 1 in 20 (5,421 per 100,000)

 The sex and age-adjusted cumulative (35 to 74 years) incremental risk of CHD mortality associated with a 1% increase (i.e.: 1.33 mmHg) in population mean SBP is approximately 1 in 2,000 (50 per 100,000). Males are more sensitive than females and constitute about 80% of the modeled CHD deaths.

Dose-Response Models

It was assumed that there is no lead effect at blood lead concentrations of 0 µg/dL and the BMC associated with an absolute increase in SBP of 1.33 mmHg and an absolute decrement in IQ of 1 point was calculated. This required extrapolating the blood lead concentration response relationship beyond (lower than) the minimum blood lead concentrations included in the critical studies to a blood lead concentration of 0 µg/dL. It was also assumed that there was no population threshold for the critical effects because the weight of evidence fails to identify a population threshold for IQ and SBP. Additionally, (1) there is evidence of significant inter-individual variability in susceptibility to the developmental neurotoxic effects and cardiovascular toxicity of lead; and (2) there is a relatively high background incidence of neurological and cardiovascular disease independent of any lead exposure. It has been suggested that inter-individual variability and background additivity will produce a low-dose linear population dose-response relationship (i.e. the response increases proportionally with an increase in dose) (Crawford and Wilson, 1996; US National Research Council, 2008).

It was not possible to fit the original study data with *de novo* blood lead concentration-response models for the IQ and SBP endpoints. Instead, the blood lead concentration-response models that were fit by the original study authors and published in the peer-reviewed literature were used for the BMC calculations. For the children's blood lead-IQ relationship, a continuous blood lead concentrationresponse model was constructed from two models published in the critical study. One of the published models was a log-linear model based on all of the study data. The second published model was a linear model based only on a subset of the study data with lower lead exposures (those subjects with a miximum blood lead concentration less than 7.5 μ g/dL). The log-linear model is not compatible with blood lead concentration-response estimates over relatively low ranges of blood lead concentrations (the slope of the log-linear model tends toward infinity as the blood lead concentration tends toward zero). Therefore, the models were joined so that the maximum slope of the log-linear model would be limited to the slope of the lower exposure linear model. The slope of the blood lead concentration-IQ relationship used in the BMD modeling is linear with a slope of -2.94 (-0.71 to -5.16) IQ points per μ g/dL below a cut-point of 0.9 (2.3 to 0.3) μ g/dL and is log-linear with a slope of -2.70 (-1.66 to -3.74) IQ points per natural log μ g/dL above the cut-point.

The regression models for the critical studies of blood concentrations and SBP are both linear models. Therefore, the adjusted regression coefficients from the published linear models can be used, without modification, as the slope parameter in the benchmark dose model for calculating risk-specific blood lead concentrations. The slopes of these models are simply extrapolated to the origin for the BMD modeling and calculations.

Benchmark Blood Lead Concentrations

The benchmark blood lead concentrations (BMCs) associated with various benchmark responses (BMRs) in mean population IQ are presented in Table EX-1. The BMCs associated with a BMR of -1 IQ point in population mean IQ are shown in bold. The best estimate BMC associated with a 1 point decrement in mean IQ is

0.3 μ g/dL, with a lower estimate BMC of 0.2 μ g/dL and an upper estimate BMC of 1.4 μ g/dL. The lower and upper estimates of the BMC reflect some, but not all of the variability and uncertainty in the slope of the blood lead concentration-IQ relationship. BMCs for other BMRs are shown for illustration.

Table EX-1. Benchmark blood lead Concentrations (BMCs) (μg/dL) corresponding to various benchmark responses (BMRs) of change in mean population IQ. BMCs are provided for the best, lower, and upper estimates of the blood lead concentration-response relationships that were derived from the international pooled analysis of Lanphear et al. (2005).

BMR (IQ points)	Lower Estimate BMC (µg/dL)	Best Estimate BMC (µg/dL)	Upper Estimate BMC (µg/dL)
-0.5	0.1	0.2	0.7
-1.0	0.2	0.3	1.4
-1.5	0.3	0.5	2.1
-2.0	0.4	0.7	2.9
-3.0	0.6	1.0	5.2
-4.0	0.8	1.5	9.6
-5.0	1.0	2.2	17.5

The benchmark blood lead concentrations (BMCs) associated with various benchmark responses (BMRs) in population mean SBP are presented in Table EX-2. The BMCs associated with a BMR of an increase in population mean SBP of 1.3 mmHg are shown in bold. The best estimate BMC associated with a 1.3 mmHg increase in population mean SBP is 2.7 μ g/dL for susceptible sub-populations and 5.0 μ g/dL for Caucasian males. These estimates are bounded by a lower estimate of 1.6 μ g/dL and an upper estimate of 25 μ g/dL. The lower and upper estimates of the BMC reflect some, but not all of the variability and uncertainty in the slope of the blood lead concentration-SBP relationship. BMCs for other BMRs are shown for illustration. Table Ex-2. Benchmark blood lead Concentrations (BMCs) (µg/dL) corresponding to various benchmark responses (BMRs) of change in mean population systolic blood pressure. BMCs are provided for the following estimates of the blood lead concentration-response relationships: lower estimate for susceptible sub-populations, best estimate for susceptible sub-populations, best estimate for Caucasian males, and an upper estimate for Caucasian males. The slopes were derived from the critical studies of Glenn et al. (2003) and Vupputuri et al. (2003).

	BMC (µg/dL):	BMC (µg/dL):	BMC (µg/dL):	
	Susceptible Sub-	Susceptible Sub-	Caucasian	BMC (µg/dL):
	populations	populations Best	Males Best	Caucasian Males
	Upper Estimate	Estimate of	Estimate of	Lower Estimate
BMR (mmHg)	of Slope	Slope	Slope	of Slope
0.5	0.6	1.1	2.0	10.0
1.0	1.3	2.1	4.0	20.0
1.3	1.6	2.7	5.0	25.0
1.5	1.9	3.2	6.0	30.0
2.0	2.5	4.3	8.0	40.0
3.0	3.8	6.4	12.0	60.0
4.0	5.0	8.5	16.0	80.0
5.0	6.3	10.6	20.0	100.0

Risk-Specific Blood Lead Concentrations

The benchmark blood lead concentrations define a single blood lead concentration associated with a specified level of response. When exposure, whether expressed as lead intake or a blood lead concentration, is measured or estimated for a risk assessment the exposure will be expressed as a distribution or as a point estimate on a distribution. To provide a useful and transparent benchmark of toxicity for risk characterization, the TRV should also be expressed as a distribution or explicitly identified as a point estimate on a distribution. Therefore, a population distribution of blood lead concentrations was assumed and the risk-specific blood lead concentrations (i.e., the TRVs) were defined as the *geometric mean* of a population blood lead distribution where the 95th percentile of that distribution is equivalent to the benchmark blood lead concentration (BMC). This allows the population risk associated with the risk-specific blood lead concentration to be described

probabilistically as the geometric blood lead concentration in a population where the health risk for 95% of the population is less than the benchmark response (BMR).

An estimate of the ratio of the 95th percentile to the geometric mean blood lead concentration was derived from analyzing the ratio of these values for nationally representative biomonitoring data. The average of the ratios was 2.88, which was then rounded to 3. BMCs were divided by 3 to calculate the risk-specific blood lead concentrations.

The risk-specific blood lead concentrations are presented in Table EX-3. The best estimate of the children's blood lead concentration where the mean lead-associated IQ decrement will be no more than 1 IQ point in 95% of the population is 0.1 μ g/dL with a lower and upper estimate of 0.1 to 0.5 μ g/dL, respectively. The best estimate of the adult blood lead concentration where the mean lead-associated increase in SBP will be no more than 1.3 mmHg in 95% of the population is 1.7 μ g/dL for Caucasian males and 0.9 μ g/dL for susceptible sub-populations. The lower and upper estimates of the adult blood lead concentration where the mean lead-associated increase in Caucasian males and 0.9 μ g/dL for susceptible sub-populations. The lower and upper estimates of the adult blood lead concentration where the mean lead-associated increase in Caucasian males and 0.9 μ g/dL for susceptible sub-populations. The lower and upper estimates of the adult blood lead concentration where the mean lead-associated increase in Caucasian males and 0.9 μ g/dL for susceptible sub-populations. The lower and upper estimates of the adult blood lead concentration where the mean lead-associated increase in CBP will be no more than 1.3 mmHg in 95% of the population are 0.5 and 6.3 μ g/dL, respectively.

	Units	Lower			
IQ		Estimate	Best Estimate	Upper Estimate	
BMR	IQ points	-1	-1	-1	
BMC	µg/dL	0.2	0.3	1.4	
Ratio 95th:GM	none	3	3	3	
Risk-specific blood lead					
concentration	µg/dL	0.1	0.1	0.5	
		Lower	Best estimate	Best estimate	Upper
SBP		estimate	susceptible	Caucasian males	estimate
BMR	mmHg	1.3	1.3	1.3	1.3
BMC	µg/dL	1.6	2.7	5	25
Ratio 95th:GM	none	3	3	3	4
Risk-specific blood lead		0.5	0.0	4.7	6.2
concentration	µg/a∟	0.5	0.9	1.7	0.3

Table EX-3. Risk-specific blood lead concentrations for the critical endpoints IQ and SBP

Cancer

The epidemiological evidence of an association between lead exposure and cancer in humans is limited, but there is sufficient evidence to conclude that lead is carcinogenic in animal experiments. There is evidence that lead is genotoxic but insufficient evidence to establish whether lead is directly genotoxic or indirectly genotoxic.

Epidemiological Evidence of the Carcinogenicity of Lead

There is limited epidemiological evidence that lead is a carcinogen. In general, epidemiological studies examined several common cancer endpoints which included lung, stomach, renal, brain and CNS, and all-site cancers. A smaller number of studies examined cancer endpoints which included thyroid, endocrine, bladder, pancreatic, prostate cancers, melanoma, lymphoma, and other cancers. This review focused on the more commonly studied cancer endpoints. Overall, the weight of evidence provides limited evidence of an association between lead exposure and cancer in epidemiological studies. The evidence for lung, stomach and all-site cancer is positive but not conclusive. The evidence for renal and brain and CNS cancer is generally more equivocal.

Experimental Evidence of the Carcinogenicity of Lead

Lead is a well established carcinogen in animal assays. There is evidence of an association between lead exposure and cancer or precancerous lesions in animals in renal, lung, and CNS tissues. At the time of this report, no animal study that was positive for stomach tumors was identified in the literature. The evidence for lung tumors is equivocal. The evidence for tumors of the CNS is positive, but limited, and the evidence for renal tumors is consistently positive.

At least 15 rat assays and at least 2 mice assays have reported significant associations between oral or subcutaneous exposure to soluble lead salts, most commonly lead acetate, and increased incidence of renal proliferative lesions, adenomas or carcinomas. Three studies, one each of mouse, hamster and rabbit, have reported no significant association between lead and renal tumors. The negative mouse and rabbit studies have methodological limitations and although no renal hyperplasia or tumors were observed in the renal tissue of the hamsters, kidney cells did show evidence of pre-neoplastic effects.

The weight of evidence supports the conclusion that soluble inorganic lead is a carcinogen in animal experiments and that the kidney is the most sensitive site of tumor occurrence in lead exposed rodents. There is also substantial evidence that lead is an effective promoter of renal tumors in rats.

Categorization of Lead as a Carcinogen

It is Health Canada policy to derive quantitative estimates of cancer potency for all CEPA Group I and II carcinogens. Lead had not previously been categorized under the CEPA criteria so that determination was made in this report.

Health Canada's criteria for categorizing carcinogens under the Canadian Environmental Protection Act (CEPA) are defined in Appendix B of the Canadian Environmental Protection Act Human Health Risk Assessment for Priority Substances (Health Canada 1994). These criteria are based on those of IARC. Health Canada's criteria for a Group II carcinogen are very similar to an IARC 2A carcinogen.

Inorganic lead meets the criteria for a Health Canada Group II carcinogen. The data from epidemiological studies is suggestive, but the strength of this evidence is generally limited by methodological shortcomings, such as imprecise exposure measurements or inadequate control of potentially confounding variables. There is strong evidence of carcinogenicity in animal species: an exposure related increase in malignant tumours has been reported in multiple species via multiple routes of exposure and in some cases, such as gliomas and renal carcinomas, the tumour site and type is unusual. A dose-response trend in the expected direction has been reported for some tumour sites in some studies; however not all studies used multiple exposure groups. There is evidence of genotoxic effects associated with lead exposure. There are several plausible Modes of Action (MOA) and associated mechanisms explaining the observed renal carcinogenicity in animals and these mechanisms are also generally relevant to humans (see below for more discussion).

Therefore, inorganic lead is classified as a Group II carcinogen according to Health Canada's CEPA carcinogen classification scheme.

Mode & Mechanisms of Carcinogenicity

There are several plausible and competing theoretical MOAs for lead associated carcinogenesis. Some of these MoAs are genotoxic (directly and indirectly by oxidative damage) while others are epigenetic. However, there is insufficient evidence to identify any single MoA as the most likely or definitive MoA and carry it forward in the analysis prescribed by the Human Relevance Framework. The default assumption, therefore, is that the MoA that is operative in the development of cancer in animals is also relevant to humans.

Critical Study for Cancer

A suitable epidemiological study to develop a quantitative estimate of the carcinogenicity of lead was not identified.

Many of the animal cancer assays for lead were performed 20 years or more ago and suffer from incomplete reporting or methodological issues. None of the available animal assays meet the 'gold standard' in animal cancer bioassays which is a chronic 2 year rodent study with rats (typically F344) and mice (typically B6C3F1) of both sexes, 50 animals per dose group, and 2 or 3 dose groups in addition to a control group.

No animal study that met all of the criteria of the gold standard in cancer bioassays was identified at the time of this report. The mouse study by Waalkes *et al.* (1995) is well designed and documented and, of the published studies reviewed, most closely

meets these criteria. The Waalkes *et al.* (1995) study deviates from these standards in a couple of important aspects: 1) the exposure was not lifetime, but perinatal only (transplacental and translactational); 2) dose groups were 23-25 animals, rather than the prescribed 50; and 3) results are reported for renal tissue only. Despite these considerations, the Waalkes *et al.* (1995) study is of sufficient quality to derive a quantitative estimate of the renal cancer potency of perinatal lead.

A second mouse study by Waalkes et al 2004 was also identified as a suitable critical study. Exposure in the second study was from weanling on.

Waalkes et al. 1995

Female C57BL/6NCr mice were bred with C3H/HeN males and were administered 0, 500, 750, and 1000 ppm lead acetate via drinking water ad libitum starting at gestational day 12 and exposure was continued until 4 weeks postpartum. Assuming the maternal mice weighed 25 g and consumed 5 ml of water per day, the maternal lead doses are equivalent to 0, 100, 150 and 200 mg/kg/d^{3.} Progeny (B6C3F1 mice) were then weaned and observed for up to 112 weeks. Litter size, body weight and survival of progeny were not affected at any of the exposure doses. Necropsies were performed on all animals. Incidence of renal proliferative lesions (RPL), including atypical tubular hyperplasia and tumors, in gestationally and lactationally exposed mice were reported as: control, 4%; 500 ppm, 16%; 750 ppm, 24%; 1000 ppm, 48%. The number of mice assessed in each exposure group was 23-25. Pre-neoplastic epithelial cell hyperplasia is often observed in association with renal cancers in

³ The authors did not measure blood Pb concentrations in the exposed mice. As a point of comparison, Fox et al. (2008) reported that exposure of rat dams to 100 ppm Pb in drinking water produced peak blood Pb of approximately 45 μ g/dL in progeny at post natal day 10.

rodents (Waalkes *et al.* 2004). Renal adenoma⁴ and cystic hyperplasia are considered precursor lesions to renal cell carcinoma. Renal tumors developed in the absence of evidence of significant concurrent lead-induced chronic nephrotoxicity. In a parallel experiment, mice with the same perinatal lead exposure were also exposed to 500 ppm of the renal tumor promoter barbital sodium (BB) in drinking water from weaning onward. Postnatal BB exposure had no significant effect on RPL incidence.

Waalkes et al. (2004)

Waalkes et al. (2004) conducted a chronic study of the renal effects of lead acetate in the drinking water of male wild-type (WT) and metallothionein-I/II knockout mice. The results from the WT mice can be used to quantify the renal cancer risk for adult only lifetime lead exposure. Comparisons to the perinatal mouse cancer assay (Waalkes et al. 1995) should be done with caution because this assay used a different strain of mice, males only and a higher dose regime. Nonetheless, the Waalkes et al. (2004) study was judged to be the highest quality study available with which to quantify the renal cancer risks from adult lifetime lead exposure. Starting at 8 weeks, mice were exposed to lead acetate in drinking water (ad libitum) at concentration of 0 (control), 1,000 ppm, 2,000 ppm, or 4,000 ppm lead. Assuming the adult mice weighed 25 g and consumed 5 ml of water per day, the lead doses are equivalent to 0, 200, 400, and 800 mg/kg/d. Mice were observed up to 112 weeks of age. Survival of WT mice was significantly reduced at the highest exposure (4,000 ppm lead). Significant depression in body weight of WT mice occurred in the 2,000 ppm (7-9% less than control) and 4,000 ppm (12-14% less than control) exposure groups. Necropsies were performed on all animals. Incidence of renal

⁴ a benign epithelial tumor of glandular origin

proliferative lesions (RPL), including atypical tubular hyperplasia and tumors, in chronically adult exposed WT mice were reported as: control, 0%; 1,000 ppm, 4%; 2,000 ppm, 12%; 4,000 ppm, 21%. Chronic adult lead exposure was not associated with a significant increase in tumors of any other tissues that were pathologically examined, including lung. Brain tissue was not examined. The incidence of hepatic tumors decreased with increasing lead dose, with a stronger protective effect reported for the metallothionein-null mice.

Quantitative Estimates of the Cancer Potency of lead

A multistage model and a linearized multistage model (LMS) were used to quantify the cancer potency of lead from two mice assays. One of the assays was for perinatal exposure only while the other was from adult exposure only. The quantitative estimates of cancer potency by all methods were higher for male mice from the preinatal exposure assay. The results discussed below have been adjusted for mice-to-human differences in kinetics. The incidence of RPLs, which is are understood to be pre-cancerous lesions, was the most sensitive endpoint measured. The adjusted TD₀₅ for RPLs in perinatally exposed male mice calculated from the multistage model is 9.2 mg/kg/d and the associated cancer unit risk (i.e. oral slope factor) is $5.46 \times 10^{-3} (mg/kg/d)^{-1}$. The TD₀₁ for the same exposure and endpoint is 2.6 mg/kg/d and the associated cancer unit risk is $1.92 \times 10^{-2} (mg/kg/d)^{-1}$. The LMS for the same exposure and endpoint produced a cancer unit risk of 1.89×10^{-2} (mg/kg/d)⁻¹. In summary, the cancer unit risks for the most sensitive sex and lifestage range from about $5 \times 10^{-3} (mg/kg/d)^{-1}$ to $2 \times 10^{-2} (mg/kg/d)^{-1}$.

As a point of comparison, using the oral slope factor of 5.46 x 10^{-3} (mg/kg/d)⁻¹ based on adjusted TD₀₅ for RPLs in perinatally exposed male mice, Health Canada's

existing pTDI for lead of 0.0036 µg/kg/d is associated with an estimated Incremental Lifetime Cancer Risk (ILCR) of approximately 2 in 100,000.

Relative Sensitivity of Cancer Endpoint

A quantitative and qualitative comparison of the relative potencies of the carcinogenic, cardiovascular, and neurotoxic effects of lead was undertaken.

- The estimated blood lead concentration associated with a 1 in 100 added risk of CHD mortality amongst susceptible subpopulations is *3 to 4 fold lower* than the estimated blood lead concentration associated with a 1 in 100 excess risk of pre-cancerous renal lesions in a susceptible subpopulation.
- The estimated blood lead concentration associated with a 1 in 100 added risk of hypertension is *50 to 1,000 fold lower* than the estimated blood lead concentration associated with a 1 in 100 excess risk of pre-cancerous renal lesions in a susceptible subpopulation.
- The estimated blood lead concentration associated with a 1 in 100 excess risk of MMR is *70 to 500 fold lower* than the estimated blood lead concentration associated with a 1 in 100 excess risk of pre-cancerous renal lesions in a susceptible subpopulation.

The weight of evidence supporting the carcinogenic effects of lead at environmentally relevant exposures is weaker than that for the developmental neurotoxicity and cardiovascular toxicity of lead.
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ACRONYMS

ACE	angiotensin I-converting enzyme
AD	Alzheimer 's Disease
ADI	Acceptable Daily Intake
AFI	Authoritarian Family Ideology
ALAD	δ-aminolevulinic acid dehydratase
ALS	Amyotrophic Lateral Sclerosis
ANCOVA	analysis of covariance
APP	ß-amyloid precursor protein
ATP1A2	sodium-potassium adenosine triphosphatase $\alpha 2$
AUC	area under the curve
BAEP	brainstem auditory evoked potential
BMC	benchmark concentrations
BMD	benchmark dose
BMI	body mass index
BMR	benchmark response
BP	blood pressure
BSID	Bayley Scales of Infant Development
CEPA	Canadian Environmental Protection Act
CHD	coronary heart disease
CHMS	Canadian Health Measures Survey
CNS	central nervous system
DA	dopamine
DMSA	meso-2,3-dimercaptosuccinic acid
DPB	Diastolic blood pressure
DRL	differential reinforcement of low rate
DSA	delayed spatial alteration
DTH	delayed type hypersensitivity

E	epinephrine
eNOS	nitric oxide synthase
ERG	electroretinograms
ET	endothelin
FAO	Food and Agriculture Organization
FBPA	
FI	fixed interval schedules of reinforcement
FR	fixed ratio schedules of reinforcement)
FSH	follicle-stimulating hormone
GAM	
GCI	
GEE	generalized estimating equations
HnRH	gonadotropin releasing hormone
HOME	Caldwell-Bradley Home Observation for Measurement of the Environment
HPA	hypothalamic-pituitary-adrenal
IARC	International Agency for Research on Cancer
JECFA	Joint Expert Committee on Food Additives
KID	Kent Infant Development Scale
LH	luteinizing hormone
LMS	linearized multistage model
LOAEL	Lowest Observed Adverse Effects Level
LRR	low range regression
LTP	long-term potentiation
MAO	monoaminooxidase
MDI	
MMR	mild mental retardation
MMSE	
ΜοΔ	mode of action

MR	mean range regression
MRL	
MSCA	McCarthy Scales of Children's Abilities
NAAQS	National Ambient Air Quality Standards
NAS	Veteran Affairs Normative Aging Study
NE	norepinephrine
NF-κB	nuclear factor kappa-beta
NHANES	National Health and Nutrition Examination Survey
NMDA	N-methyl-D-aspartate
NMNG	N-methyl-N'-nitro-N-nitrosoguanidine
NO	nitric oxide
NOAEL	No Observed Adverse Effects Level
PD	Parkinson's Disease
PDI	Psychomotor Development Index
PKC	protein kinase C
PND	postnatal day
PNS	peripheral nervous system
POD	point of departure (POD)
pTDI	provisional tolerable daily intake
RBC	red blood cell
RfC	
RfD	
RPL	renal proliferative lesions
RPLs	renal proliferative lesions
SAP	Schneider Neonatal Assessment for Primates
SBP	systolic blood pressure
SCE	sister-chromatid exchange
SE	standard error

SES	socioeconomic status
тс	
TC05	tumorigenic concentration 05
TD ₀₅	tumorigenic dose 05
TDI	
TDL05	tumorigenic dose low 05
TPR	total peripheral vascular resistance
TRV	toxicological reference value
VDR	vitamin D receptor
VEP	visual evoked potential
VI	variable interval schedules of reinforcement
WHO	World Health Organization
WISC	Wechsler Intelligence Scales for Children
WISC-R	Wechsler Intelligence Scale for Children-Revised
WT	wild-type

Glossary

Adjustment Factor: Synonymous with uncertainty factor.

Benchmark Dose (BMD) or Concentration (BMC): A dose or concentration that produces a predetermined change in response rate of an adverse effect (called the benchmark response or BMR) compared to background (US EPA, 2007b). Defined within the range of the dose-response data (i.e., via data interpolation).

BMDL or BMCL: A statistical lower confidence limit on the dose or concentration at the BMD or BMC, respectively (US EPA, 2007b).

Benchmark Response (BMR): An adverse effect, used to define a benchmark dose. The change in response rate over background of the BMR is usually in the range of 5-10%, which is the limit of quantification of responses typically observed in well-conducted animal experiments (US EPA, 2007b).

Critical Effect: is the first biologically significant adverse effect expected to occur as exposure dose or concentration increases above zero (Health Canada, 1994; US EPA, 2007b).

Critical Study: The study that contributes most significantly to the qualitative and quantitative assessment of hazard. Also called Principal Study (US EPA, 2007b), pivotal study, key study.

Dose: The amount of a substance available for interactions with metabolic processes or biologically significant receptors after crossing the outer boundary of an organism. The potential dose is the amount ingested, inhaled, or applied to the skin. The applied dose is the amount presented to an absorption barrier and available for absorption (although not necessarily having yet crossed the outer

boundary of the organism). The absorbed dose is the amount crossing a specific absorption barrier (e.g. the exchange boundaries of the skin, lung, and digestive tract) through uptake processes. Internal is a more general term denoting the amount absorbed without respect to specific absorption barriers or exchange boundaries. The amount of the chemical available for interaction by any particular organ or cell is termed the delivered or biologically effective dose for that organ or cell. (US EPA, 2007b)

Dose-Response Assessment: A determination of the relationship between the magnitude of an administered, applied, or internal dose and a specific biological response. Response can be expressed as measured or observed incidence or change in level of response, percent response in groups of subjects (or populations), or the probability of occurrence or change in level of response within a population. (US EPA, 2007b).

Dose-Response Relationship: The relationship between a quantified exposure (dose) and the proportion of subjects demonstrating specific biologically significant changes in incidence and/or in degree of change (response) (US EPA, 2007b).

Hazard Characterization: A description of the potential adverse health effects attributable to a specific environmental agent, the mechanisms by which agents exert their toxic effects, and the associated dose, route, duration, and timing of exposure (US EPA, 2007b).

Hypertension: A chronic medical condition of elevated blood pressure classified by three stages of severity: Pre-Hypertension: SPB >130 mmHg Stage I Hypertension: SBP > 140 mmHg

Stage II Hypertension: SBP > 160 mmHg

Key Study: See Critical Study.

Lowest-Observed-Adverse-Effect Level (LOAEL): The lowest exposure level at which there are biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control group (US EPA, 2007b).

No-Observed-Adverse-Effect Level (NOAEL): The highest exposure level at which there are no biologically significant increases in the frequency or severity of adverse effect between the exposed population and its appropriate control; some effects may be produced at this level, but they are not considered adverse or precursors of adverse effects (US EPA, 2007b).

Non-Threshold Toxicant: Substances for which the critical effect is assumed to have no threshold (i.e. traditionally restricted to mutagenic carcinogens). It is assumed that there is some probability of harm at any level of exposure and it is not appropriate to calculate a dose below which adverse effects are not expected to occur (Health Canada, 1994).

Parity: The condition or fact of having borne offspring

Pivotal Study: See Critical Study.

Point of Departure: The dose-response point that marks the beginning of a lowdose extrapolation. This point can be the lower bound on dose for an estimated incidence or a change in response level from a dose-response model (BMD), or a NOAEL or LOAEL for an observed incidence, or change in level of response (US EPA, 2007b).

Principal Study: See Critical Study.

Provisional Tolerable Daily Intake (pTDI): a TDI associated with sufficient uncertainty that is likely to be updated or will knowingly require updating, in the future.

Reference Value (RfV): An estimate of an exposure for a given duration to the human population (including susceptible subgroups) that is likely to be without an appreciable risk of adverse health effects over a lifetime. It is derived from a BMDL, a NOAEL, a LOAEL, or another suitable point of departure, with uncertainty/variability factors applied to reflect limitations of the data used. (US EPA, 2007b)

Threshold Toxicant: Substances for which the critical effect is not cancer or a heritable mutation. It is assumed that there is some level of exposure below which adverse effects are not expected to occur (Health Canada, 1994).

Tolerable Concentration (TC): airborne (or waterborne) concentration of a chemical, expressed in units mass per volume air (i.e. mg chemical/m³) (or mass per volume water; mg/L), to which it is believed that a person can be exposed daily over a lifetime without deleterious effect (Health Canada, 1996).

Tolerable Daily Intake (TDI): Daily intake by ingestion, normalized for total body weight, expressed in units of mass of chemical per mass of body weight per day (i.e.

mg/kg body weight/day), to which it is believed that a person can be exposed daily over a lifetime without deleterious effect (Health Canada, 1996).

Tumorigenic Concentration 05 (TC₀₅): airborne (or waterborne) concentration of a chemical, expressed in units mass per volume air (i.e. mg chemical/m³) (or mass per volume water; mg/L), which induces a 5% increase in the incidence of, or deaths due to, exposure-related tumours (Health Canada, 1996).

Tumorigenic Dose 05 (TD₀₅): Daily intake by ingestion, normalized for total body weight, expressed in units of mass of chemical per mass of body weight per day (i.e. mg/kg body weight/day) which induces a 5% increase in the incidence of, or deaths due to, exposure-related tumours (Health Canada, 1996).

Uncertainty/Variability Factor (UFs): Synonymous with **Adjustment Factor**. One of several, generally 10-fold, default factors used in operationally deriving the TRV from experimental data. The factors are intended to account for (1) variation in susceptibility among the members of the human population (i.e., inter-individual or intraspecies variability); (2) uncertainty in extrapolating animal data to humans (i.e., interspecies uncertainty); (3) uncertainty in extrapolating from data obtained in a study with less-than-lifetime exposure (i.e., extrapolating from subchronic to chronic exposure); (4) uncertainty in extrapolation when the database is incomplete, particularly with regard to categories of health outcomes or target tissues or organs that have not been adequately investigated. (US EPA, 2007b)

SECTION 1 • INTRODUCTION

This report presents a critical review of the evidence of the toxicological effects of lead (Pb) and recommends toxicological reference values (TRVs) for primary prevention of adverse population health effects from environmental lead exposure in Canada.

The lead TRVs presented in this report are not intended to define the maximum amount of environmental lead exposure that is *tolerable* or *acceptable*. The lead TRVs presented in this report are points of *reference* to help risk assessors quantify the potential health risks associated with a given lead exposure scenario and describe the associated uncertainty. Whether or not the potential health risks are *tolerable* or *acceptable* is a value judgement that is within the purview of risk managers and stakeholders.

This report has three general parts: (1) an overview of the diverse systemic toxic effects of lead and a comparison of the relative weight of evidence for adverse effects on specific systems or endpoints at relatively low blood lead concentrations; (2) detailed reviews of the evidence for three "critical" effects: developmental neurotoxicity, toxicity to the vascular system, and cancer; and (3) the derivation of TRVs for these critical effects.

TRVs for non-cancer effects are traditionally based on the assumption that a threshold of toxicity can be identified and the TRV is less than the putative threshold. In the case of lead, however, no threshold can currently be identified for developmental neurotoxicity, vascular toxicity and several other systemic effects. Additionally, the US National Research Council, several publications by researchers and risk assessors, and some referees of an earlier draft of this

report recommended that TRVs for non-carcinogens, and lead in particular, be based on the assumption that there is no threshold for population health effects. Under this alternative framework, the slope of the dose-response relationship is estimated and a probabilistic risk-specific dose is calculated from this slope. Accordingly, this report provides recommended slopes for the relationship between blood lead concentrations and IQ deficits in children and for the relationship between blood lead concentrations and increased systolic blood pressure (SBP) in adults. These slopes are extrapolated to the origin to derive risk-specific blood lead concentrations for population effects on IQ and SBP.

Several TRVs for the carcinogenic effects of lead are derived. These include: (1) risk-specific doses and associated unit risks using a multistage model; and (2) unit risks using a linearized multistage model.

This section of the report provides background information on why the report was undertaken and the purpose of the report, describes some of the general methods used in the preparation of the report, presents an outline of the remaining report sections, and identifies some important limitations to the TRVs derived in this report.

1.1 BACKGROUND

Toxicological Reference Values are Benchmarks for Primary Prevention

Toxicological Reference Value (TRV) is a generic term that includes more narrowly defined terms for both non-carcinogenic substances (such as Tolerable Daily Intake (TDI), Tolerable Concentration (TC) Reference Dose (RfD), Reference Concentration (RfC), Minimum Risk Level (MRL), Acceptable Daily Intake (ADI), and their equivalents) and carcinogens (such as cancer slope factor, q_1^* , risk specific dose, risk specific concentration and their equivalents).

Threshold TRVs assume that there is some level of exposure that is without adverse health effects. As far back as 1962 the World Health Organization defined a threshold TRV (Acceptable Daily Intake) as "the daily intake of a chemical, which during an entire lifetime appears to be without appreciable risk on the basis of all known facts at that time" (WHO, 1962). Current definitions of threshold TRVs used by many health agencies differ little from this basic concept (Health Canada, 1994; US EPA, 2002).

Non-threshold TRVs have traditionally been used for mutagenic carcinogens (and carcinogens of uncertain mode of action). Non-threshold TRVs assume that there is some probability of effect associated with any dose. Therefore, non-threshold TRVs provide a quantitative estimate of risk. Over the years there have been calls from risk assessors and academics for health agencies to extend this approach to non-carcinogens that have no apparent population threshold (Crawford and Wilson, 1996; Crump *et al.*, 1997; Gaylor *et al.*, 1999; Clewell and Crump, 2005)

TRVs may be generically described as benchmark values used by risk assessors to help characterize the degree of risk presented by an environmental exposure scenario. TRVs are also used as the toxicological benchmark inherent in various environmental quality guidelines and regulations. By both these applications, TRVs are used to help determine the allowable amount of environmental exposure to potentially toxic substances. In this context, TRVs may be viewed as toxicological benchmarks for the primary prevention of population health effects from environmental exposures to toxic substances.

Blood Lead Intervention Values are not Toxicological Reference Values

The TRVs for lead derived in this report are not synonymous with blood lead intervention values and are not intended to replace them.

Health Canada recommends individual and community lead exposure intervention strategies on the basis of measured blood lead values (CEOH, 1994). The lowest of the currently recommended blood lead intervention values is 10 µg/dL (0.48 µmol/L), established in Canada in 1994. This value is consistent with the lowest blood lead level of concern defined by the US Centres for Disease Control and Prevention (US CDC) in 1991. Health Canada's blood lead intervention values are currently under review.

It is important to note that the lowest blood lead intervention value is not a TRV for primary prevention. However, in the absence of a lead TRV expressed as a blood lead concentration, the lowest blood lead intervention value has been used as a TRV – both in site specific risk assessment and in the derivation of numeric environmental quality criteria. The blood lead intervention values and strategies are analogous to numeric environmental quality criteria in that they are derived on the basis of considering both health effects and risk management issues, such as feasibility, cost, benefits, and health risks at an individual level. Both Health Canada (CEOH, 1994) and the US CDC have explicitly stated that the lowest blood lead intervention values are not toxicological thresholds (US Centres for Disease Control and Prevention, 1991). Multiple publications since 1991 by the

US CDC and members of the Advisory Committee on Childhood Lead Poisoning Prevention have emphasized that 10 μ g/dL does not define a threshold for the harmful effects of lead (Binns *et al.*, 2007).

1.2 HEALTH CANADA'S CURRENT TRV FOR LEAD

Health Canada has not previously derived its own TRV for lead. Most programmes within Health Canada currently use the provisional tolerable weekly intake (pTWI) for lead or the daily equivalent recommended by the World Health Organization and Food and Agriculture Organization (WHO/FAO) Joint Expert Committee on Food Additives (JECFA) (WHO, 1986; WHO, 1993; WHO, 1995; WHO, 2000). This TRV was most recently reviewed and upheld by JECFA in 1999. The daily equivalent of the JECFA pTWI is a provisional tolerable daily intake (pTDI) of 3.6 μ g/kg/d. Unpublished research conducted by Health Canada in preparation of this report indicates that this TRV is widely used around the world. In fact, of the health agencies contacted in several dozen countries, all were using either the JECFA pTWI (or equivalent) or the lowest blood lead intervention level of 10 μ g/dL as their TRVs for environmental lead exposure.

The pTWI was originally derived to prevent an unacceptable accumulation of lead in blood. However, recent advances in the understanding of lead toxicokinetics mean that there is considerable uncertainty in the blood and bone lead concentrations associated with chronic ingestion of lead at the pTWI. Also, recent epidemiological and toxicological literature on the health effects of lead suggest that blood lead concentrations that were previously considered safe may be associated with risk of adverse health effects. Taken together, these developments raise uncertainties about the level of health protection that is afforded by the existing pTWI.

1.3 HISTORY OF PARALLEL DECLINES IN LEAD EXPOSURE AND REFERENCE VALUES

Average blood lead concentrations in western nations, including Canada, have declined by about 10-fold since the 1970s. As blood lead concentrations have declined scientists have been able to study the potential toxic effects of lead at ever decreasing concentrations. This has resulted in a continued history of "discovering" that lead exposure levels that were previously thought to be safe, or without risk of harm, are associated with adverse health effects.

As blood lead concentrations and estimates of toxic thresholds have declined, so too has government policy on what constitutes a maximally tolerable lead intake and blood lead concentration. The US blood lead intervention level declined sixfold from 1970 to 1991, but has remained constant since. A similar history of declines has occurred in Canada. The Royal Commission on Lead in the Environment in the 1970s recommended a blood lead concentration of concern of 25 μ g/dL based on an understanding that a threshold for the haematopoietic effects of lead was at 40 μ g/dL. In 1994 the lowest individual blood lead intervention level in Canada was set to 10 μ g/dL. The history of the lowest blood intervention level relative to historical average blood lead concentrations is illustrated in Figure 1.



Figure 1. Lowest US Blood Lead Intervention Levels and Average Blood Lead Concentrations in North American Children

Note: Blood lead concentration data are from US NHANES, except for 2008 data from CHMS. Canadian lead intervention levels have historically been similar to those in the United States.

The maximum daily permissible intake of lead recommended by an expert committee of the US Public Health Service in 1971 was 300 μ g/d (equivalent to about 20 μ g/kg/d for a toddler) (King, 1971). At the time it was thought that intakes less than this amount would not result in the accumulation of lead in a child's body. In 1986 JECFA established a tolerable weekly intake of 25 μ g/kg (equivalent to 3.6 μ g/kg/d) based on the understanding that this lower level of intake also would not result in the accumulation of lead in a child's body. In 1999 JECFA upheld this tolerable weekly intake based on the understanding that it would not produce a blood lead concentration in children greater than 10 μ g/dL.

The lowest blood lead intervention level has declined six-fold since the 1960s, yet the recommended maximum daily intake from all sources has only declined three-fold over the same period and no change in the recommended maximum daily intake has been made since the mid 1980s.

There are no known physiological requirements for or functions of lead (it is not an essential element). Estimates of pre-metallurgical environmental levels and human body burdens of lead (i.e., prior to the Bronze Age) are 500-1,000 fold lower than today. While blood lead concentrations in western nations have declined about 10-fold since their peak in 1950s-1970s, contemporary blood lead concentrations are estimated to be at least 100-fold higher than natural background levels (Ericson *et al.*, 1979; Ericson *et al.*, 1991; Wittmers *et al.*, 2008).

1.3.1 History of Health Canada Contractor Reports

Recent scientific developments raise questions about the degree of health protection inherent in the pTWI for lead (on a population basis) as well as the lowest blood lead intervention value (on an individual basis). In preparation for a review of both the pTWI and the blood lead intervention values, Health Canada commissioned a series of contractor reports. These reports are identified and briefly summarized below.

Wilson et al. (2005)

Wilson *et al.* (2005) undertook a critical review of the basis of Health Canada's pTDI for lead. The report found that the pTDI is based on the JECFA pTWI and that the pTWI: (1) was originally based on metabolic studies that indicated that a daily oral intake of 3-4 μ g/kg/d was not associated with an increase in blood lead concentration; and (2) was later based on an interpretation of 10 μ g/dL as a

threshold of effects. The report concluded that Health Canada's Provisional Tolerable Daily Intake pTDI for lead is associated with an uncertain blood lead concentration and may not protect against adverse health effects.

Cantox Environmental Inc. et al. (2006)

Cantox Environmental Inc. *et al.* (2006) was commissioned at the same time as Wilson *et al.* (2005) and provided a review of the epidemiological evidence of the association between blood lead concentrations < 10 μ g/dL and developmental neurotoxicity. The report concluded that the epidemiological evidence supports an association between blood lead concentrations < 10 μ g/dL and neurocognitive effects in children. No threshold of effects was identified. The report concluded that the weight of evidence should be used to develop a reference concentration for blood lead - a concentration below which adverse effects are thought not to occur or are considered negligible. Cantox Environmental Inc. *et al.* (2006) was peer reviewed by external referees.

Equilibrium Environmental Inc. (2008a)

Equilibrium Environmental Inc. (2008a) was commissioned in response to the reports by Wilson *et al.* (2005) and Cantox Environmental Inc. *et al.* (2006). The purpose of the Equilibrium Environmental Inc. (2008a) report was to conduct a review of the toxicological effects of lead and to recommend methods for the development of reference biomarker (blood lead and bone lead) concentrations. The report recommended reference biomarker concentrations be developed on the basis of the developmental neurotoxicity and the cardiovascular toxicity of lead and that, in the absence of an identified threshold for these critical effects, the reference biomarker concentrations be based on a Benchmark Dose (BMD)

approach. Equilibrium Environmental Inc. (2008a) was peer reviewed by external and internal referees.

Equilibrium Environmental Inc. (2008b)

Equilibrium Environmental Inc. (2008b) was commissioned in anticipation of the development of reference biomarker concentrations for blood and bone lead. The purpose of the report was to evaluate the toxicokinetic literature on lead and existing toxicokinetic models for lead and recommend a model or models that best characterize the relationship between chronic absorbed dose and blood and bone lead concentrations. The modeled relationship between chronic absorbed dose and biomarker concentrations was examined in isolation to avoid the complexity and potential confusion from the simultaneous consideration of intake, bioavailability and toxicokinetics. The report recommended that either the US EPA IEUBK or the O'Flaherty model be used to model the relationship between chronic absorbed dose and blood lead concentrations in children. The report recommended that the O'Flaherty model be used to model the relationship between chronic absorbed dose and blood lead and cortical bone lead concentrations in adults. The report further recommended that a three-fold adjustment factor be used to account for the cumulative uncertainty in environmental lead concentrations, intake, bioavailability and toxicokinetics. Equilibrium Environmental Inc. (2008b) was guided by an international panel of external peer collaborators.

Summary of Contractor Reports

The overall conclusions and recommendations from this series of contractor reports are:

- Health Canada's existing pTDI for lead (3.6 µg/kg/d) is associated with an uncertain blood lead concentration
- There is evidence of adverse health effects at blood lead concentrations less than the lowest intervention value of 10 µg/dL
- There is a requirement to define a reference blood lead concentration associated with no adverse effects or minimally acceptable adverse effects
- The relationship between blood lead concentrations in children and chronic absorbed dose (uptake) can currently be most defensibly modeled by either the IEUBK or the O'Flaherty toxicokinetic models for lead
- The relationship between blood and cortical lead concentrations in adults and chronic absorbed dose (uptake) can currently be most defensibly modeled by the O'Flaherty toxicokinetic model for lead
- A three-fold adjustment factor should be used to account for the cumulative uncertainty in interrelationships among environmental lead concentrations, intake, bioavailability and toxicokinetics.

This report was prepared in response to the findings of these contractor reports. The purpose of this report is to provide updated TRVs to guide decision-making about primary prevention of population health effects associated with environmental lead exposure and to provide these TRVs as blood lead concentrations. A separate report addresses the requirement for updated guidance on individual blood lead interventions.

1.4 METHODS

This section provides a general overview of methods that were common to all sections of this report. More specific descriptions of methods are provided in the relevant sections of the report.

Blood and Bone Lead as the Exposure Metric

In much of the literature on lead health effects, exposure is expressed as a biomarker concentration - most commonly blood lead and more recently bone lead. For this reason, the health effects of lead are better understood in the context of associated blood or bone lead concentrations, rather than external dose or environmental concentrations.

Lead has a relatively short residence time in blood and it accumulates in bone. For example, about 90% of the adult body burden of lead is found in the skeleton. Therefore, bone lead concentrations potentially provide a superior estimate of cumulative lead exposure and may be more predictive of the chronic health effects of lead than blood lead concentrations. However, it is only within the last decade or so that the availability and precision of non-invasive *in vivo* x-ray fluorescence (XRF) bone lead measurements has improved to the point that the technique has been used in epidemiological studies of environmental cohorts, with literature on the relationships between bone lead and health effects only recently emerging.

Tiered Approach to the Review of Evidence

The review of toxicological evidence followed a tiered approach. This was done as a matter of efficiency and it is not left that this tiered approach significantly affects the overall conclusions of the review nor alters the final TRVs that have been derived from it.

Tier I Evidence for Identification of Critical Effects

An exhaustive search and review of all of the primary literature was not undertaken to identify the candidate critical effects of lead. As a first tier, third party reviews were consulted to develop a general understanding of the biomarker-response relationships for each of the major biological systems or organs affected by lead. Third party reviews that were consulted for this evidence included the Health Canada commissioned peer reviewed contractor reports listed above, recently published peer reviewed toxicological reviews of lead by other health agencies, and recently published review articles in the peer reviewed literature.

Tier II Evidence for Identification of Critical Effects

For each of the identified critical effects, a systematic search of the available English language literature on the epidemiological, *in vivo* and *in vitro* evidence was conducted. Primary searches were conducted in the MEDLINE PubMed database using relevant MeSH terms and the TOXLINE database using key words. The results of the database searches were verified by cross referencing literature cited in recently published review articles. The cut-off publication date for systematic searches was December 31, 2007. However, current awareness was maintained by using Ovid Current Content alerts and included any relevant papers published prior to October 1, 2009.

1.5 REPORT OUTLINE

The report has three general parts: (1) an overview of the diverse systemic toxic effects of lead and a comparison of the relative weight of evidence for adverse effects on specific systems or endpoints at relatively low blood lead concentrations; (2) detailed reviews of the evidence for three "critical" effects: developmental neurotoxicity, toxicity to the vascular system, and cancer; and (3) the derivation of TRVs for these critical effects. These three parts are covered in the following report sections.

Section 2 of the report provides an overview of the relative weight of evidence for the adverse effects of lead on various organ systems. The purpose of this section is to illustrate the wide variety of adverse effects associated with relatively low blood lead concentrations and the relative weight of evidence behind these effects. A second objective is to identify "critical" effects that will be carried forward for TRV derivation. Threshold TRVs are traditionally developed on the basis of a critical effect – which is defined as the adverse effect evoked by the lowest exposure concentration. However, because so many of the systemic effects of lead are without an identified threshold, a critical effect by this definition cannot be identified. Instead, the critical effects were defined as: (1) those endpoints or systematic effects that have the greatest relative weight of evidence at the lowest blood lead concentrations; and (2) those outcomes for which a blood lead concentration-response relationship can be characterized with the greatest relative certainty. The critical effects identified by these criteria are IQ in children and systolic blood pressure in adults. Cancer was also identified as a critical effect for TRV derivation because of policy considerations.

Section 3 of the report provides a detailed review of the observational and experimental evidence of the developmental neurotoxicity of lead. Within this domain, the relationship between children's blood lead concentrations and decrements in school-aged IQ was identified as the biomarker-response relationship that could be characterized with the most certainty and was, therefore, carried forward for TRV development.

Section 4 of the report provides a detailed review of the observational and experimental evidence of the cardiovascular toxicity of lead. Within this domain, the relationship between adult blood lead concentrations and systolic blood pressure was identified as the biomarker-response relationship that could be characterized with the most certainty and was, therefore, carried forward for TRV development.

Section 5 of the report provides estimates of the slope of the relationship between blood lead concentrations and IQ and the slope of the relationship between systolic blood pressure (SBP). These estimates are based on epidemiological studies of the blood lead concentration-response relationships over ranges of lead exposure that are close to or within the range of current environmental lead exposures for most Canadians. The estimated slopes are provided so that risk assessors can calculate the incremental changes in population health endpoints associated with incremental changes in blood lead concentrations that are within the range of current environmental lead exposures.

Section 6 of the report provides risk-specific blood lead concentrations for population effects on IQ and SBP. This requires that the estimates of the blood lead concentration-response slopes for IQ and SBP be extrapolated below the range of study data. The slopes are extrapolated to the origin so that risks

associated with lead exposures that are less than the critical study data (and current blood lead concentrations) can be estimated. Extrapolation is necessary for risk assessors who must characterize potential health benefits associated with lowering current environmental lead exposures. The following risk-specific blood lead concentrations were calculated: (1) the average blood lead concentration associated with no more than a 1 point average decrement in IQ among 95% of the population; and (2) the average blood lead concentration associated no more than a 1.3 mmHg average increase in SBP among 95% of the population. The risk-specific blood lead concentrations may be used as points of reference to help determine acceptable or tolerable lead exposure risks, but they are not intended to be benchmarks of acceptable or tolerable risks.

Section 7 of the report provides a detailed review of the evidence of the carcinogenic effects of lead, categorizes lead according to the criteria for categorization of carcinogens under the Canadian Environmental Protection Act (CEPA), identifies suitable critical studies to derive quantitative estimates of cancer potency, and presents several cancer unit risks and risk-specific doses based on various methods. A quantitative and qualitative comparison is also made between cancer and non-cancer health risks.

1.6 LIMITATIONS

The following important limitations to this report should be noted.

Absence of health risks at or below the TRVs should not be expected

It is important to recognize that all TRVs, including those derived for lead herein, are estimates. There is a great deal of scientific uncertainty, natural variability, and professional judgement inherent in any TRV. No TRV should be interpreted as a *bright line* demarking exposures that are without effect from those that are. Rather, TRVs should be viewed as a *point of reference* on a continuum of probability of adverse effects. The probability of adverse health effects will rise in proportion to the degree to which exposures exceed the TRV and, conversely, the probability of adverse health effects will decrease in proportion to the degree to which exposures are less than the TRV.

Apportionment

The TRVs for lead derived herein are for the primary prevention of health effects from all environmental sources of chronic and subchronic lead exposure. Chronic exposure is defined as three months of exposure or longer and subchronic exposure is defined as greater than a single dose but less than chronic. In the absence of toxicological data on the health effects of subchronic exposure, it is recommended that the chronic TRVs also be used to assess the potential health risks from subchronic exposures. There is insufficient toxicological data to differentiate the magnitude of adverse health effects that may arise from subchronic versus chronic exposures at the levels of exposure equivalent to the TRVs derived herein. Therefore, all sources of chronic and subchronic environmental lead exposure must be accounted for in assessing risks against these TRVs.

Occupational Lead Exposures
It is recognized that employees that may be occupationally exposed to lead are under the jurisdiction of a regulatory framework that typically offers some additional level of environmental monitoring and medical surveillance over and above that that is provided to the general public. This additional protection may warrant the use of different TRVs for the assessment and management of occupational lead exposures.

SECTION 2 • BLOOD LEAD CONCENTRATIONS AND HEALTH EFFECTS

The purpose of this section is to provide an overall summary of the evidence of the toxic effects of lead, as measured by blood lead concentrations, on various organs and systems. A secondary objective is to identify the "critical effects" that will be carried forward for derivation of Toxicological Reference Values (TRVs).

Information on the potential health effects of lead exposure is expressed in terms of blood lead concentrations because this is the exposure metric that is most commonly used in both experimental and observational studies of the toxic effects of lead.

Definition of Critical Effect

TRVs are derived on the basis of a quantified dose-response relationship for a single health endpoint. Traditionally, this approach has been justified by ensuring that the TRV is derived on the basis of the "critical", or most sensitive, effect. A critical effect is defined as the first biologically significant adverse effect expected to occur as exposure dose or concentration increases above zero (Health Canada, 1994; US EPA, 2007b). However, in the case of lead there is insufficient evidence to identify any single effect as critical by the strict definition of the term. To do so would require an understanding of the relative thresholds for the various adverse effects of lead – something that is currently absent.

As an alternative, the critical effects for lead were defined as: (1) those endpoints or systematic effects that have the greatest relative weight of evidence at the lowest blood lead concentrations; and (2) those outcomes for which a blood lead concentration-response relationship can be characterized with the greatest relative certainty. These endpoints are:

- Early life blood lead concentrations and deficits in IQ and
- Adult blood lead concentrations and increased systolic blood pressure (SBP).

Justification for the selection of these critical effects is provided below. It is emphasized that these are not the only endpoints for which there is evidence of adverse effects associated with relatively low blood lead concentrations. Risk assessors should keep in mind that these endpoints, to a degree, are surrogates for all of the other potential health effects associated with a given blood lead concentration.

2.1 METHODS

This section will present the methods used to collect, interpret, and summarize data on the various organ systems affected by lead exposure and the blood lead concentrations at which there is evidence for adverse effects.

The results of the summaries are presented in two formats:

- *Narrative toxicological summaries.* A narrative summary was developed for each of the major organ systems adversely affected by lead.
- A blood lead weight of evidence heuristic model. This model presents an integrated graphical representation of all of the narrative toxicological summaries.

Sources of Data

Third party, peer reviewed critical reviews were relied upon as a starting point for data. Third party sources of information consulted to characterize the blood lead-response relationships for candidate critical effects included:

- The risk assessment conducted by the US EPA in support of the updated National Ambient Air Quality Standard for Lead (US EPA, 2006)
- The International Agency for Research on Cancer Monograph on the Evaluation of Carcinogenic Risks to Humans for inorganic and organic lead compounds (IARC, 2006)
- The US Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profile for Lead (US ATSDR, 2007)
- The US National Toxicology Program (NTP) background document on Lead for its Report on Carcinogens, 11th Edition. (U.S. Department of Health and Human Services, 2004)
- The World Health Organization (WHO) International Programme on Chemical Safety's (IPCS) Environmental Health Criteria monograph on Lead (WHO, 1995)
- Systematic critical reviews, pooled analyses and meta-analyses on various toxicological effects of lead published in English in the peer reviewed literature.

And the following contractor reports on Lead recently commissioned by Health Canada:

 Critical Review of Potential Health Effects Associated with Lead (Pb) (Equilibrium Environmental Inc., 2008a)

- Update of Evidence for Low-Level Effects of Lead and Blood Lead Intervention Levels (Cantox Environmental Inc. *et al.*, 2006)
- Lead (Pb) Risk Assessment in Canada, Part I: Critical Review of Toxicity Reference Values (Wilson *et al.*, 2005).

The primary literature was consulted as required for verification, clarification, or to include more recently published reports. The cut-off date for literature searches for this section of the report was October 1, 2009.

Scope

This report focuses on the most commonly studied endpoints and the summary information presented here is not exhaustive. For example, data on adverse impacts on the auditory and hepatic systems are not presented.

The scope of data used to develop the toxicological summaries was limited to that where exposure was expressed as a blood lead concentration. The great majority of studies, especially more recent publications, on the toxicological effects of lead quantify lead exposure as a biomarker concentration – most often blood or bone lead concentrations, but also occasionally plasma or serum lead concentrations. Of these, blood lead is by far the most commonly used biomarker of lead exposure.

There are very few data on the relationship between blood lead concentrations in experimental animals and cancer. The blood lead concentrations associated with oral doses from key cancer bioassays were estimated on the basis of blood lead concentrations achieved in other rodent studies with similar exposure magnitude, duration and timing.

Weight of Evidence Heuristic

A heuristic model was developed as a means to present information on the weight of evidence of adverse effects at various blood lead concentrations. A heuristic is an aid or guide to problem solving and not necessarily an exact solution. The intent of the model is to present a sufficient representation of the evidence to facilitate relative comparisons across endpoints at a fairly gross level of resolution. Readers are cautioned against making precise inferences or reaching definitive conclusions based solely on this model.

Data were abstracted from original papers or review articles to identify the lowest blood lead concentration associated with an effect or the highest blood lead concentration associated with no effect. The evidence of adverse effects was categorized as:

- Strongly positive
- Moderately positive
- Suggestive or
- Strongly Negative

The criteria presented in Table 1 were used to categorize the evidence:

Weight of Evidence	Epidemiological	In vivo
Strongly positive	Multiple studies, consistently positive	Positive findings in multiple studies
Moderately positive	Multiple studies, predominantly positive	Single positive study
Suggestive	Single positive study	Multiple studies, equivocal findings
Strongly negative	Multiple studies, predominantly negative	Multiple studies, predominantly negative

 Table 1. Criteria for categorizing evidence of effects

The criteria used to categorize the weight of evidence were initially developed to account for evidence from human observational (epidemiological) studies, experiments with laboratory animals (*in vivo*), and *in vitro* experimental evidence. In the end, however, quantitative *in vitro* experimental evidence is not presented in the model because of the uncertainties inherent in calculating an *in vivo* blood lead equivalent to *in vitro* exposure concentrations and because most *in vitro* experiments are not based on chronic exposure.

The experimental and observational evidence was reviewed for each endpoint. The range of blood lead concentrations associated with each category of evidence was identified. The critical decision in this step was identifying the lowest blood lead concentration associated with an individual category of evidence (or in the case of negative evidence, the highest associated blood lead concentration). Because studies vary in how they report a significant result (positive or negative), a series of rules were developed to guide the selection of a blood lead concentration associated with a reported result.

Depending on whether blood lead was measured as a continuous or categorical variable, the following rules were developed to guide the selection of a blood lead concentration associated with the evidence.

Categorical

No (or Lowest) Observed Adverse Effects Level (N/LOAEL). NOAEL or LOAELs are usually the mean blood lead concentration of a categorical exposure or dose group. In this case, the group mean or median blood lead concentration associated with the NOAEL or LOAEL was selected. Where no measure of central tendency was given for a categorical blood lead group and an effect was detected, the minimum of the group range was selected for a LOAEL and if no effects was detected, the maximum of the group range was selected for a NOAEL. Any other categorical comparisons, between quartiles for example, were also treated as a N/LOAEL equivalent, even if it was not stated as such in the original publication.

Continuous

A benchmark dose is the blood lead concentration associated with a specified benchmark response (BMR) on an interpolated blood lead concentration-response curve. In this case, the reported BMD value, not a lower confidence limit on the BMD, was selected. In a few cases, the level of response was defined as specified inhibitory concentration (IC_X) – these were interpreted to beequivalent to a BMD.

Regression model. In this case various statistics associated with the distribution of blood lead data may be reported as the upper or lower blood lead associated with the effect. As a default, the mean or other measure of central tendency of the range of blood lead concentrations were selected.. This was defined as the mean range regression, or MRR. In cases where authors conducted a threshold analysis or otherwise demonstrated that the blood lead concentration-response relationship extended to the lower range of the study data, the minimum (or maximum in the case of negative results), of the range of blood lead concentrations included in the regression model was selected. These values were defined as the low range regression (LRR).

Data for the blood lead weight of evidence heuristic model was recorded in data summary tables. The following information was abstracted for each study included in the data summary table: study type (epidemiological or *in vivo*), the lowest blood lead concentration associated with a positive result or the highest blood lead concentration associated with a negative result, the method of assigning a blood lead concentration, the measurement endpoint for the study, and the reference for the study.

The data summary tables are not intended to present an exhaustive listing of all studies reviewed for a particular endpoint, rather they are meant to provide sufficient evidence that the criteria for a particular category of weight of evidence has been met. For example, the criteria for "moderately positive" evidence are: (1) mostly positive multiple epidemiological studies; or (2) a single positive animal study. Under this category the single animal study with the lowest blood lead concentration associated with a positive response were listed or, where the preponderance of results were positive, the two epidemiological studies associated with the lowest blood lead concentrations.

2.2 RESULTS

Narrative toxicological summaries are presented below for each of the major organ systems adversely affected by lead. The narrative summaries also include the data summary tables that were used to generate the blood lead weight of evidence heuristic model. The model provides a snap-shot of the relative weight of evidence for each of the major organs or systems affected by lead.

2.2.1 Developmental Neurotoxic Effects

Developmental neurotoxicity is the most intensively researched of all of lead's adverse outcomes. The weight of evidence from observational, *in vivo* and *in vitro* studies supports the conclusions that:

- Developmental neurotoxicity has been associated with the lowest levels of lead exposure examined, both in observational studies and in animal experiments
- The neurotoxic effects of lead have been shown to persist long after exposures have ceased and blood and brain lead concentrations have returned to normal or control levels
- The preponderance of data from observational studies do not show any evidence of a population threshold for developmental neurotoxicity over the lower ranges of current environmental lead exposures. For some endpoints, such as IQ deficits, the preponderance of evidence indicates that the dose-response relationship is curvilinear, with a steeper slope over the lower ranges of current environmental lead exposures.
- Lead has been shown to interact with all cell types in the central nervous system and there are multiple plausible modes of action supported by experimental evidence to explain the observed developmental neurotoxicity of lead and these modes of action are relevant in humans. Cellular and biochemical perturbations are caused by lead *in vitro* at exposure concentrations that are up to 100-fold lower than the equivalent whole blood lead concentrations associated with current environmental exposures.

Epidemiological studies have reported an association, after adjusting for confounders, between early life lead exposure and adverse developmental effects on a variety neurological endpoints including:

- Neuromotor function (Dietrich *et al.*, 1993a; Wasserman *et al.*, 2000b; Ris *et al.*, 2004; Despres *et al.*, 2005; Fraser *et al.*, 2006)
- Academic achievement and reading or math skills (Needleman *et al.*, 1990; Fergusson *et al.*, 1997; Lanphear *et al.*, 2000; Al-Saleh *et al.*, 2001; Wang *et al.*, 2002; Miranda *et al.*, 2006; Chandramouli *et al.*, 2009);
- Delinquent or antisocial behaviour (Fergusson et al., 1993; Bellinger et al., 1994b; Needleman et al., 1996; Dietrich et al., 2001; Needleman et al., 2002)
- Attention and executive function (Bellinger *et al.*, 1994a; Canfield *et al.*, 2003b; Chiodo *et al.*, 2004; Ris *et al.*, 2004; Braun *et al.*, 2006; Chiodo *et al.*, 2007)
- Auditory function (Schwartz and Otto, 1991; Dietrich et al., 1992; Osman et al., 1999)
- Visual function (Fox *et al.*, 1997; Fox *et al.*, 2008; Laughlin *et al.*, 2008Rothenberg *et al.*, 2002b)

Many of these effects have been associated with blood lead concentrations less than 10 μ g/dL (Osman *et al.*, 1999; Lanphear *et al.*, 2000; Canfield *et al.*, 2003a; Canfield *et al.*, 2003b; Chiodo *et al.*, 2004; Despres *et al.*, 2005; Fraser *et al.*, 2006; Miranda *et al.*, 2006; Chiodo *et al.*, 2007) and most, but not all (Chandramouli *et al.*, 2009), report a dose-response relationship that extends down to the lowest blood lead concentrations studied (1-2 μ g/dL). However, the endpoint that has been most studied and for which there is the greatest weight of evidence of a causal relationship is the adverse consequences of early life lead exposure on psychometric tests of intelligence (IQ) among school-aged children. The epidemiological evidence is strongly suggestive, but not consistently supportive, of an association between early life chronic lead exposure (as measured by various biomarkers) and decrements in school-aged children's IQ. However, where studies of early life lead exposure and childhood IQ have failed to reach statistical significance, the direction of effect has generally been supportive of an effect (Ernhart et al., 1987; Cooney et al., 1989a; Cooney et al., 1989b; Ernhart et al., 1989; Bellinger et al., 1991; Cooney et al., 1991; Bellinger et al., 1992; Dietrich et al., 1992; Schnaas et al., 2006). Many epidemiological studies provide evidence of a lack of threshold for developmental neurotoxicity down to the lowest blood lead concentrations measured in their studies – in the range of 1-2 µg/dL (Schwartz, 1994a; Lanphear et al., 2000; Canfield et al., 2003a; Chiodo et al., 2004; Lanphear et al., 2005; Schnaas et al., 2006; Tellez-Rojo et al., 2006; Chiodo et al., 2007), but not all studies report this pattern of results (Surkan et al., 2007). Considered in the context of potential measurement error, the potential for over control of modifying, rather than confounding variables, the relative insensitivity of IQ as a measure of cognitive injury, and the complex dependence of effects on magnitude, duration, and timing of exposure, the overall pattern of findings from the observational literature is relatively strong and clear.

There is evidence that the developmental neurotoxic effects associated with childhood blood lead concentrations persist out to at least the late teen-age years (Fergusson *et al.*, 1997; Ris *et al.*, 2004).

A vast literature from experimental toxicology supports the conclusions of the observational studies. There is no equivalent to an IQ test in animals, but

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adverse behavioural outcomes that reflect learning and memory have been demonstrated at the lowest blood lead concentrations studied (about 10 µg/dL) in multiple species, including non-human primates (Cory-Slechta and Thompson, 1979; Rice, 1984a; Rice, 1985; Rice and Gilbert, 1985; Gilbert and Rice, 1987; Rice and Karpinski, 1988). Animal studies demonstrate that the developmental neurotoxicity of lead persists after exposures are halted and blood and brain lead concentrations return to normal. No threshold for lead induced behavioural deficits in laboratory animals has been established.

Lead has been shown to interact with all cell types in the central nervous system. Several c exposure concentrations as low as the picomolar range. The equivalent *in vivo* whole blood lead concentrations are uncertain, but are estimated to be up to 100-fold lower than those produced by contemporary environmental lead exposures in Canada.

Neither observational nor experimental data have established a "critical window" nor a "signature effect" for the developmental neurotoxicity of lead. In addition to the functional deficits associated with lead exposure, experimental and observational studies have shown lead to be associated with structural abnormalities and physiological impairments.

In summary, multiple epidemiological studies report an association between early life blood lead concentrations and adverse neurological development at blood lead concentrations as low as 1-2 μ g/dL; these findings, however, are not 100% unequivocal. At least one cross-sectional study reports an inverse association between maternal blood lead concentrations over the range of 0.28 to 1.18 μ g/dL and their offspring's scores on tests of infant intelligence (Emory *et al.*, 2003). Multiple animal studies report neurobehavioural effects associated with

developmental lead exposure producing blood lead concentrations of 10 to 12 $\mu\text{g}/\text{dL}.$

The relevant studies for determining the blood lead weight of evidence heuristic for neurodevelopmental effects are presented in Table 2.

	Blood Pb					
	associated					
		with				
Туре	Outcome	outcome	Units	Metric	Reference	
Epi	IQ decrements in children 5-10 yrs	0.5	µg/dL	LRR	Lanphear <i>et al</i> 2005	
	IQ decrements and other neurological impairment in children	1	µg/dL	LRR	Chiodo <i>et al</i> 2004	
	Decrements in Bayley MDI in 2 yr olds	1	µg/dL	LRR	Tellez-Rojo <i>et al</i> 2006	
	Decreased score on Fagan Test of Infant Intelligence (FTII),	1.18	µg/dL	LOAEL	Emory <i>et al</i> 2003	
	Decrements in Bayley MDI in 2 & 3 yr olds	1.23	µg/dL	MRR	Jedrychowski <i>et al</i> 2009	
	Decreased performance on standardized tests of reading in math in grade 4	2	µg/dL	LOAEL	Miranda <i>et al</i> 2006	
	IQ decrements in 3-5 yr olds	2.4	µg/dL	LRR	Canfield et al 2003	
	IQ decrements in 10 yr olds	2.5	µg/dL	LRR	Schnaas <i>et al</i> 2006	
	Decreased math and reading skills in 6-16 yr olds	<5	µg/dL	Group	Lanphear <i>et al</i> 2000	
	IQ decrements in 10 yr olds	7	µg/dL	MRR	Bellinger et al 1992	
	IQ decrements in 11-13 yr olds	7.9	µg/dL	MRR	Tong <i>et al</i> 1996	
	Decreased performance on tests of standardized educational outcomes	5-10	µg/dL	LOAEL	Chandramouli <i>et al</i> 2009	
	IQ decrements in children	5-10	µg/dL	LOAEL	Surkan <i>et al</i> 2007	
	No IQ decrements in children	3-4	µg/dL	NOAEL	Surkan <i>et al</i> 2007	
	No effect on tests of standardized educational outcomes	2-5	µg/dL	NOAEL	Chandramouli <i>et al</i> 2009	
In vivo	Neurobehavioural impairments in rats	10	µg/dL	LOAEL	Cory-Slechta <i>et al</i> 1979	
	Neurobehavioural impairments in monkeys	12	µg/dL	NOAEL	Gilbert <i>et al</i> 1987; Rice 1984b, 1985; Rice <i>et al.</i> 1985, 1988	

Table 2. Weight of evidence summary table for neurodevelopmental effects of lead.

1. NOAEL: no observed adverse effects level; LOAEL: lowest observed adverse effects level; MRR: mid-range of

significant multiple regression model; LRR: minimum range of significant multiple regression model with evidence of nonlinearity; IC50: inhibitory concentration 50

2.2.2 Neurodegenerative Effects

Neurodegenerative effects have not been as well studied as developmental neurotoxicity. The observational and experimental evidence that do exists, though, support an association between lead exposure and increased rate of neurological decline. Associations between bone lead concentrations and neurodegenerative effects have been more consistently reported than associations between blood lead and neurodegenerative effects (Shih *et al.*, 2006).

The risk of scoring less than 24 on the Mini-Mental State Exam (MMSE) was inversely associated with blood lead concentrations among subjects of the Normative Aging Study (NAS) with mean blood lead concentrations of 5 μ g/dL (Wright *et al.*, 2003). These results, however, are in contrast to findings from other cohort studies with similar levels of exposure (Muldoon *et al.*, 1996; Nordberg *et al.*, 2000; Krieg *et al.*, 2005). Tibia lead, but not blood lead was associated with cognitive decline in a cohort of older women with blood lead concentrations of 2.9 μ g/dL (Weuve *et al.*, 2009). Tibia lead, but not blood lead, was also associated with current cognitive performance and cognitive decline in a longitudinal occupational cohort study (Khalil *et al.*, 2009).

The δ -aminolevulinic acid dehydratase (ALAD) genotype may modify the relationship between lead exposure and cognitive decline (Rajan *et al.*, 2008). In a case-control study, cases of essential tremor (ET) had higher blood lead concentrations and there was an interaction between blood lead concentration and ALAD allele status; the risk of ET was 30 fold higher in those with higher blood lead concentrations and homozygous for the ALAD2 allele (Louis *et al.*, 2005).

There is inconsistent epidemiological evidence of an association between lead and Amyotrophic Lateral Sclerosis (ALS). A recent cohort study reported longer survival times were associated with blood lead and bone lead concentrations in patients diagnosed with ALS, with patella lead having a much stronger effect (Kamel *et al.*, 2008). Bone lead, but not blood lead, was associated with current cognitive performance and cognitive decline in a longitudinal occupational cohort study (Khalil *et al.*, 2009).

Experimental evidence from rats and non-human primates has also shown early life blood lead concentrations of about 20-30 µg/dL and biochemical and pathological effects that are characteristic of Alzheimer 's disease (AD) (Basha *et al.*, 2005; Wu *et al.*, 2008); the putative mechanism for these effects, altered DNA methylation, has been associated with environmental lead exposure in an observational study (Pilsner *et al.*, 2009). At the time of this report, no environmental cohort studies of lead and AD were located in the literature; the observational data from occupational exposures are equivocal (Graves *et al.*, 1991), but suffer from methodological limitations.

Occupational cohort studies have relatively consistently reported a number of central nervous system (CNS) and peripheral nervous system (PNS) effects at blood lead concentrations above about 20 µg/dL, with few data at lower blood lead concentrations. Effects reported in occupational cohort studies include abnormal postural sway, abnormal visual evoked potential (VEP) and brainstem auditory evoked potential (BAEP), peripheral sensory nerve impairment, neuromotor impairment, and neurological symptoms.

There are few experimental data on the effects of lead-induced neurodegeneration on animal behaviour. Studies in both rats and non-human

primates at blood lead concentrations of about 20 µg/dL demonstrate that older animals are more susceptible to the adverse neurobehavioural effects of lead than younger animals with comparative dose and duration of exposure (Rice, 1990; Cory-Slechta and Pokora, 1991; Rice, 1992a; Rice, 1992c).

In summary, there is suggestive but inconsistent evidence of an association between blood lead concentrations as low as about 5 µg/dL and neurodegeneration in older, environmentally exposed people. Bone lead has been more consistently associated with neurodegeneration than blood lead. Experimental evidence shows that early life lead exposure as low as about 12 µg/dL can result in AD-like neurochemical changes and pathology (Wu *et al.*, 2008), but this effect has not been shown in humans. Occupational cohort studies and experimental evidence provide relatively consistent evidence of adverse effects on the PNS and CNS in adults at blood lead concentrations greater than about 20 µg/dL.

The relevant studies for determining the blood lead weight of evidence heuristic for neurodegenerative effects are presented in Table 3.

		Blood Pb			
		associated			
		with			
Туре	Endpoint	outcome	Units	Metric	Reference
Epi	Increased risk for essential tremor (30x greater risk for ALAD2)	3	µg/dL	LOAEL	Louis <i>et al</i> 2003
	No association with neuropsychological test scores	3.3	µg/dL	MRR	Krieg <i>et al.</i> 2005
	Increased risk of MMSE score < 24	3.4	µg/dL	LOAEL	Wright <i>et al</i> 2003
	No association with MMSE score	3.7	µg/dL	MRR	Nordberg <i>et al</i> 2000
	No association with neuropsychological test scores (urban)	5.4	µg/dL	MRR	Muldoon <i>et al</i> 1996
	Impaired neuropsychological test scores (rural)	7	µg/dL	LOAEL	Muldoon <i>et al</i> 1996
In vivo	Neurobehavioural impairments in adult monkeys	11	µg/dL	LOAEL	Rice <i>et al</i> 1988
	Increased beta-amyloid proteins in older rats exposed only as infants	20	µg/dL	LOAEL	Basha <i>et al</i> 2005
	Neurobehavioural impairments (increased	20	µg/dL	NOAEL	Cory-Slechta
					<i>et al</i> 1991a
	Neurobehavioural impairments in adult monkeys	25	µg/dL	LOAEL	Rice, 1990, 1992b, 1992c
	Increased beta-amyloid proteins and plaques in 23 year old monkeys exposed only as infants	32-36	µg/dL	LOAEL	Wu <i>et al</i> 2008

Table 3. Weight of evidence summary table for neurodegenerative effects of lead.

1. NOAEL: no observed adverse effects level; LOAEL: lowest observed adverse effects level; MRR: mid-range of significant multiple regression model; LRR: minimum range of significant multiple regression model with evidence of non-linearity; IC50: inhibitory concentration 50

2.2.3 Cardiovascular Effects

The hypertensive effects of lead have been well studied in both humans and animals. The observational data is inconsistent, but suffers many methodological issues. There is also evidence of several modifiers of effect, such as age, sex, and genetics that make it more difficult to discern a clear signal in observational studies. Older animal data at higher exposures is also inconsistent. However, more recent low-level experimental evidence has consistently shown that blood lead concentrations as low as $3-5 \ \mu g/dL$ causes significantly elevated blood pressure in rodents and there is evidence of several relevant modes of action. The weight of evidence conclusion is that lead has toxic effects on the vascular system at environmental exposures.

Lead exposure has been associated with several cardiovascular endpoints, including cardiovascular mortality, stroke mortality, myocardial infarction (MIA) mortality, inotropic and chronotropic cardiotoxicity, and peripheral arterial disease and there is evidence for several of these effects at blood lead concentrations less than 10 µg/dL (Lustberg, 2002; Navas-Acien *et al.*, 2004; Menke *et al.*, 2006; Schober *et al.*, 2006; Navas-Acien *et al.*, 2007; Navas-Acien *et al.*, 2008). However, the endpoints that have been most studied and for which there is the greatest weight of evidence of a causal relationship are lead induced increases in blood pressure, particularly systolic blood pressure (SBP), or risk of hypertension.

There have been a large number of epidemiological studies of blood lead and blood pressure or risk of hypertension in adults and many, but not all, report a significant, but modest association. Several meta-analyses also report a significantly positive association. The modest effect size and the inconsistency of results can be attributed, in part, to measurement error in both lead exposure and blood pressure. In contrast to studies that have relied on blood lead as a measure of exposure, bone lead has more consistently been associated with increased blood pressure or risk of hypertension. The few observational data on the cardiovascular effects of lead in children are inconsistent. Observational data suggest that there are subpopulations that may be more susceptible to the hypertensive effects of lead. These potentially susceptible subpopulations include pregnant women, African-Americans, subjects with elevated psychological stress, and carriers of certain genetic polymorphisms. The effect size for these susceptible subpopulations may be ten-fold higher.

Lead exposure has been associated with increased SBP or risk of hypertension among environmental cohorts with average blood lead concentrations as low as 3-5 µg/dL (Vupputuri *et al.*, 2003; Martin *et al.*, 2006). There have been few analyses of the shape of the blood lead concentration-response relationship for cardiovascular effects; some show evidence of an attenuation of the slope over lower ranges of current blood lead concentrations for SBP and MIA mortality (Schwartz *et al.*, 2000a; Martin *et al.*, 2006; Menke *et al.*, 2006), but not stroke mortality (Menke *et al.*, 2006).

Both chronic and subchronic oral exposure to lead in laboratory animals results in elevated blood pressure. This effect has been demonstrated in multiple species and has been repeatedly demonstrated in multiple rat strains – three week oral lead exposures that produce a mean blood lead concentration of 2.4 μ g/dL result in about a 30% increase in blood pressure in rats.

The collective evidence from epidemiological studies and *in vivo* and *in vitro* experimental studies clearly indicate that there are several mechanisms by which chronic lead exposure could cause elevated blood pressure and related cardiovascular disease. The mode of action for which there is the most evidence is vasoconstriction secondary to lead-induced oxidative stress and subsequent

inactivation of the vasodilator nitric oxide (NO) and related signalling pathways and functional responses.

In summary, there is suggestive, but not entirely consistent, epidemiological evidence of an association between environmental lead exposure and increased SBP or risk of hypertension among subjects with average blood lead concentrations as low as about 3-5 μ g/dL. However, experimental evidence has consistently shown that low level lead exposures producing blood lead concentrations of 3-5 μ g/dL cause elevated blood pressure and several relevant modes of action have been described.

The relevant studies for determining the blood lead weight of evidence heuristic for cardiovascular effects are presented in Table 4.

		Blood Pb					
		associated					
		with					
Туре	Outcome	outcome	Units	Metric	Reference		
Epi	Increased risk of cardiovascular mortality	3.6	µg/dL	LOAEL	Menke <i>et al</i> 2006		
	Stress induced increase in total	2.8	µg/dL	LOAEL	Gump <i>et al</i>		
	children			2005			
	Increased risk of peripheral arterial	2.9	µg/dL	LOAEL	Navas-Acien		
	disease				<i>et al</i> 2004		
	Increased SBP among African- Americans	3.4-5.4	µg/dL	MRR	Vupputuri <i>et</i> <i>al</i> 2003		
	Increased SBP among Caucasian	4.6	µg/dL	MRR	Glenn <i>et al</i>		
	IIIdies				2003		
	No association with ambulatory	2.9	µg/dL	MRR	Staesson <i>et al</i>		
	501				1996		
In vivo	Increased blood pressure in rat	2.4	µg/dL	LOAEL	Attri <i>et al</i> 2003		
	Increased blood pressure in rat	3.2	µg/dL	LOAEL	Ding <i>et al</i> 1998		

Table 4. Weight of evidence summary table for cardiovascular effects of lead.

1. NOAEL: no observed adverse effects level; LOAEL: lowest observed adverse effects level; MRR: mid-range of significant multiple regression model; LRR: minimum range of significant multiple regression model with evidence of non-linearity; IC50: inhibitory concentration 50

2.2.4 Haematopoietic Effects

Lead has adverse effects on erythrocyte morphology and lifespan and haeme synthesis. Blood lead concentrations typical of contemporary environmental exposures are associated with c commonly associated with blood lead concentrations in excess of about 40 µg/dL, although these adverse effects are dependent on age and iron status and have been reported at lower blood lead concentrations in young or iron deficient subjects.

Changes in haematopoietic enzymes and substrates have been observed at the lowest blood lead concentrations measured in epidemiological studies. Lead inhibits several haematopoietic enzymes and epidemiological studies have reported decreased blood ALAD activity and increased urinary δ -aminoaluvulinic acid associated with blood lead concentrations in cohorts with blood lead concentrations of 15-20 µg/dL are associated with a 50% decrease in blood ALAD activity in both children and adults. Increased erythrocyte protoporphyrin (or zinc protoporphyrin) was historically used as a biomarker of lead exposure but is only associated with blood concentrations in excess of about 20 µg/dL. The threshold for effects on erythrocyte protoporphyrin is lower in iron deficient children (Marcus and Schwartz, 1987).

Thresholds for clinically significant haematological effects (anaemia, depressed haemoglobin) of lead are dependent on both age and iron status. Anaemia becomes more common at blood lead concentrations > 40 μ g/dL in children and > 50 μ g/dL in adults. A 10% probability of anaemia (defined as haematocrit < 35%) in children was associated with a blood lead concentration of 20 μ g/dL at 1 year of age, 50 μ g/dL at three years of age, and 75 μ g/dL at five years of age (Marcus and Schwartz, 1987). It was estimated from an occupational cohort study that a blood lead concentration of 20 μ g/dL is associated with a 5% excess risk of abnormal levels of haemoglobin and red blood cell (RBC) count; a blood lead concentration of 30 μ g/dL is associated with a 5% excess risk of abnormal levels of haemoglobin and red blood cell (RBC) count; a blood lead concentration of 30 μ g/dL is associated with a 5% excess risk of abnormal levels of haemoglobin and red blood cell (RBC) count; a blood lead concentration of 30 μ g/dL is associated with a 5% excess risk of abnormal levels of haemoglobin and red blood cell (RBC) count; a blood lead concentration of 30 μ g/dL is associated with a 5% excess risk of abnormal levels of haemoglobin and red blood cell (RBC) count; a blood lead concentration of 30 μ g/dL is associated with a 5% excess risk of abnormal levels of haemoglobin and red blood cell (RBC) count; a blood lead concentration of 30 μ g/dL is associated with a 5% excess risk of abnormal levels of haemoglobin and red blood cell (RBC) count; a blood lead concentration of 30 μ g/dL is associated with a 5% excess risk of abnormal levels of haemoglobin and red blood cell (RBC) count; a blood levels of haemotocrit (Karita *et al.*, 2005).

There are relatively few *in vivo* studies of the haematopoietic effects of lead. A study by lavicoli *et al.* (2003) reported a decreased RBC count in mice at blood

lead concentrations of 7-13 μ g/dL. Zareba and Chmielnick (1992) reported that blood lead concentrations of 17.5 μ g/dL in rabbits resulted in inhibition of ALAD.

There is experimental and epidemiological evidence that lead can decrease survival, alter the morphology, and perturb the energenics of erythrocytes. Altered erythrocyte morphology and function has been reported in a human cohort study with average blood lead concentrations of $3.3 \ \mu g/dL$ (Jacob *et al.*, 2000). There is *in vitro* evidence of lead-induced alteration of the erythrocyte membrane at lead concentrations of $0.1 \ \mu M$ (equivalent to whole blood lead concentration of about $2 \ \mu g/dL$).

In summary, the epidemiological evidence is suggestive down to a geometric mean blood lead concentration of 3 µg/dL (Jacob et al., 2000). Blood lead concentrations of 15-20 µg/dL have repeatedly been associated with a 50% decrease in blood ALAD activity; the blood lead concentrations in these study cohorts ranged down to 2 to 5 µg/dL (Hernberg et al., 1970; Morita et al., 1997). A single in vivo experiment demonstrated decreased RBC in mice with blood lead concentrations of 7-13 µg/dL (lavicoli et al., 2003). This same study reported increased RBC, haemoglobin and haematocrit in mice with blood lead concentrations less than 2 µg/dL, but these results have not been replicated. Decreased blood ALAD activity was reported in rats with blood lead concentrations of 10 µg/dL (Terayama et al., 1986). Therefore, there is suggestive evidence of adverse haematological effects of lead down to a blood lead concentration of 3 µg/dL, moderate evidence at 7 µg/dL and strong evidence at 10 µg/dL. It was estimated from an occupational cohort study that a blood lead concentration of 20 µg/dL is associated with a 5% excess risk of abnormal levels of haemoglobin and RBC count; a blood lead concentration of 30 µg/dL is associated with a 5% excess risk of abnormal levels of haematocrit. There is no

strong evidence of a threshold for the lead-associated changes in haematopoietic enzymes or precursors, although there is suggestive evidence for a biphasic dose-response curve for some haematopoietic effects.

The relevant studies for determining the blood lead weight of evidence heuristic for haematopoietic effects are presented in Table 5.

		Blood Pb			
		associated			
		with			
Туре	Outcome	Outcome	Units	Metric	Reference
Epi	Altered erythrocyte morphology and function	3.3	µg/dL	MRR	Jacob <i>et al.</i> 2000
	Decreased blood ALAD activity	16	µg/dL	IC50	Hernberg <i>et</i> <i>al</i> . 1970
	Decreased blood ALAD activity	20.1	µg/dL	IC50	Mortia <i>et al.</i> 1997
In vivo	Increased RBC count in mice	2	µg/dL	LOAEL	lavicoli <i>et al</i> 2003
	Decreased RBC count in mice	7	µg/dL	LOAEL	lavicoli <i>et</i> <i>al</i> 2003
	Decreased blood ALAD activity in rats	10	µg/dL	LOAEL	Terayama <i>et al</i> 1986
	Decreased blood ALAD activity in rabbits	17.5	µg/dL	LOAEL	Zareba <i>et al</i> 1992

Table 5. Weight of evidence summary table for haematopoietic effects of lead.

1. NOAEL: no observed adverse effects level; LOAEL: lowest observed adverse effects level; MRR: mid-range of significant multiple regression model; LRR: minimum range of significant multiple regression model with evidence of non-linearity; IC_{50} : inhibitory concentration 50

2.2.5 Reproductive Effects

Lead is an endocrine disruptor that acts on multiple sites in both the male and female hypothalamic-pituitary-gonadal axis.

Male Reproductive System

The *in vivo* evidence is often contradictory. Lead-induced male reproductive effects are dependent on dose, duration and timing of exposure. The biological cycling of testosterone secretion rates in rats and monkeys may explain some of the apparent inconsistencies because this periodicity, if not controlled for, adds anouther source of variability in the potential response of the male reproductive system.

Relatively high developmental lead exposure can delay the onset of male puberty and alter reproductive function. Lead concentrations in excess of about 30 μ g/dL *in vivo* in male animals have been associated with delayed sexual maturation, reduced semen quality, morphological and histological changes in sex organs, and impaired *in vivo* fertilization. The studies are inconsistent, though,with respect to the dose and timing of exposure required to elicit these reproductive effects.

Changes in male sex hormones and cellular structure are associated with lower blood lead concentrations. Blood lead concentrations as low as 20 µg/dL in monkeys suppressed gonadotropin releasing hormone (GnRH)-induced secretion of luteinizing hormone (LH), decreased testosterone:LH, and altered Sertoli cell function (depressed inhibin:follicle-stimulating hormone (FSH) ratios) (Foster *et al.*, 1993). Subchronic lead exposure in adult rats that produced blood lead concentrations of about 7 µg/dL resulted in structural effects in Sertoli cells and spermatids and reduced numbers of spermatozoa (Barratt *et al.*, 1989; Murthy *et al.*, 1995). Blood lead concentrations of 20 µg/dL in weanling rats also resulted in structural changes to Sertoli cells (Murthy *et al.*, 1995). Blood lead concentrations of 14 µg/dL in rats were associated with structural damage to seminiferous tubules and reduced number of prospermatogonia (Corpas *et al.*, 1995). The

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evidence for effects on these endpoints at these blood lead concentrations is inconsistent.

Decreased sperm count and morphological aberrations have most often been reported in occupationally exposed men with blood lead concentrations > 40 μ g/dL. A 50% inhibitory concentration (IC₅₀) for reduced sperm was estimated at 50 μ g/dL (Bonde *et al.*, 2002). A single occupational cohort study reported that sperm concentrations were inversely associated with blood lead concentrations as low as 15 μ g/dL (Alexander *et al.*, 1996). Decreased sperm concentration, motility, morphology, and viability were correlated with lead concentrations in seminal fluid or lead in spermatozoa, but not with blood lead in a cohort with mean blood lead concentrations of about 9 μ g/dL. There is only one environmental cohort study on sperm quality and it is not clear that the suggested threshold for adverse effects on sperm from occupational studies is a true threshold or a reflection of the exposure ranges that have been studied.

There is evidence that time to pregnancy is correlated with male blood lead concentrations down to at least as low as 10 μ g/dL, although the results are inconsistent. Shiau *et al.* (2004) reported that time to pregnancy increased by 0.15 months for every μ g/dL increase in blood lead between 10 and 40 μ g/dL. Blood lead concentrations in occupationally exposed males of 10-20 μ g/dL were associated with about a 25% (8-51%) increase in risk of infertility; an increased risk of 50% (8-200%) was associated with blood lead concentrations of 40-50 μ g/dL (Sallmen *et al.*, 2000).

It must be noted that many of the epidemiological studies on male fertility did not carefully measure or adjust for confounding variables.

In summary, increased risk of infertility and increased time to pregnancy have been associated with male occupational blood lead concentrations as low as 10 μ g/dL. Some but not all *in vivo* studies report perturbed sex hormones, morphological aberrations, and decreased sperm quantity or quality at blood at lead concentrations as low as 7 to 10 μ g/dL. Blood lead concentrations in excess of about 30 μ g/dL *in vivo* in male animals have been associated with delayed sexual maturation, reduced semen quality, morphological and histological changes in sex organs, and impaired *in vivo* fertilization.

There is a relatively high degree of inconsistency in both the experimental and epidemiological literature. This may be due to the periodicity of testosterone secretion rates in test species, effect modification by timing or duration of exposure, and imprecision of both measures of endpoint and exposure and the potential influence of confounding variables in epidemiological studies. There is also a paucity of epidemiological investigations on reproductive effects in environmentally exposed males.

Female Reproductive System

Lead delays sexual maturation in females, and there is epidemiological evidence of delayed puberty in adolescent girls associated with blood lead concentrations as low as 3 μ g/dL (Selevan *et al.*, 2003). These findings have been replicated in two of the three additional studies that have examined this effect (Wu *et al.*, 2003; Denham *et al.*, 2005; Wolff *et al.*, 2008) and are supported by recent and replicated *in vivo* studies that reported a 30% delay in onset of puberty in mice with blood lead concentrations of 2.3 μ g/dL compared to mice with blood lead concentrations of 0.7 μ g/dL (lavicoli *et al.*, 2004; lavicoli *et al.*, 2006). These low dose *in vivo* studies also report a supralinear dose-response relationship, with no evidence of an attenuation in the relationship as blood lead concentrations approach 0.7 μ g/dL. Delayed onset of puberty and reductions in circulating levels of insulin-like growth factor 1 (IGF₁), luteinizing hormone (LH), and estradiol (E₂) were associated with rats exposed during gestation only with a peak blood lead concentration of about 14 μ g/dL (Dearth *et al.*, 2002). Altered levels of progesterone were reported in monkeys with blood lead concentrations of 25-30 μ g/dL (Foster *et al.*, 1996). At blood lead concentrations greater than about 40 μ g/dL in animal experiments, lead alters menstrual cycles and the morphology of the placenta and impairs fertility and reproductive success.

Older epidemiological studies report an association between spontaneous abortion and occupational lead exposure. Findings from more recent environmental cohort studies are inconsistent, but methodological issues limit the strength of conclusions that can be made from them. There is one well designed, but not yet replicated study that reports a concentration-effect gradient over blood lead concentrations of 3 to 29 μ g/dL with an increased odds ratio of spontaneous abortion of 1.8 (1.1-3.1) per 5 μ g/dL increase in blood lead concentration (Borja-Aburto *et al.* (1999). There are few epidemiological data on lead and female fertility or fecundity.

Survival and Morphology of Offspring

Lead can result in developmental toxicity to offspring at maternal blood lead concentrations that do not produce maternal clinical toxicity. Effects range from fetal mortality to reduced birth weight and postnatal growth.

In vivo fetal mortality and reduced birth weight and postnatal growth have been associated with maternal blood lead concentrations of 30-50 µg/dL in rats and

monkeys. Maternal blood lead concentrations of 13 μ g/dL have resulted in increased resorptions in dams and maternal blood lead concentrations of 10 μ g/dL have resulted in increased external malformations in pups (Flora and Tandon, 1987).

The epidemiological evidence of an association between maternal blood lead and birth weight and pre-term birth are inconsistent. Gonzalez-Cossio *et al.* (1997) reported that infants from mothers with tibia lead in the highest quartile (> 15 μ g/g) were, on average, 156 g lighter than infants from mothers in the lowest quartile (<4.5 μ g/g). Hernandez-Avila *et al.* (2002) also reported an association between tibia lead and shorter birth length in this cohort.

Summary of Reproductive Effects

Observational studies have reported delays in onset of male and female puberty associated with blood lead concentrations as low as 3 μ g/dL. These findings are supported by replicated *in vivo* studies, that report delays in onset of female puberty down to blood lead concentrations as low as 0.7 μ g/dL. The low dose *in vivo* studies also report a supralinear dose-response relationship. On the strength of this, there is strong evidence of reproductive effects as low as 3 μ g/dL and moderately strong evidence of effects as low as 0.7 μ g/dL. The replicated *in vivo* findings of an effect down to 0.7 μ g/dL were not categorized as strong because the replicated results are from the same laboratory. The findings of effects on other developmental endpoints are more difficult to interpret. There are relatively few epidemiological data on reproductive endpoints and those which exist are predominantly from occupationally exposed cohorts. Data from both epidemiological and experimental studies tend to be conflicting. The inconsistency may be due to relatively small effect sizes, inter-individual

variability in response, imprecision in measures of exposure and effect, or due to methodological issues. As previously mentioned, the inconsistency in results among experimental studies may also be attributed to the periodicity of testosterone secretion in rats and monkeys. A single environmental cohort study reports that the risk of spontaneous abortion increases in a dose-dependent manner above blood lead concentrations of 3 μ g/dL. Increased risk of infertility and increased time to pregnancy has been associated with blood lead concentrations above 10 μ g/dL in occupationally exposed males. There is relatively consistent epidemiological evidence that blood lead concentrations in excess of about 40 μ g/dL are associated with decreased sperm count.

The relevant studies for determining the blood lead weight of evidence heuristic for reproductive effects are presented in Table 6.

Blood Pb						
		associated				
with						
Туре	Outcome	outcome	Units	Metric	Reference	
Epi	Delayed puberty in females	3	µg/dL	LOAEL	Selevan <i>et al</i> 2003	
	Delayed puberty in females	3	µg/dL	LOAEL	Wu <i>et al</i> 2003	
	Delayed puberty in males	3	µg/dL	LOAEL	Hauser <i>et al</i> 2008	
	Increased risk of spontaneous abortion	3	µg/dL	LRR	Borja-Aburto <i>et</i> <i>al</i> 1999	
	Increased time to pregnancy	10	µg/dL	LRR	Shiau <i>et al</i>	
					2004	
	Increased relative risk of	10-15	µg/dL	Group range	Sallmenn <i>et al</i>	
	intertility				2000	
	Decreased sperm concentrations	15-24	µg/dL	Group range	Alexander <i>et al</i> 1996	
In vivo	Delayed onset of puberty in mice	1.7	µg/dL	LOAEL	lavicoli <i>et al</i> 2006	
	Delayed onset of puberty in mice	1.7	µg/dL	LOAEL	lavicoli <i>et al</i> 2004	
	Altered structure of Sertoli cells and spermatids	7	µg/dL	LOAEL	Murthy <i>et al</i> 1995	
	Decreased spermatozoa	7	µg/dL	LOAEL	Barratt <i>et al</i> . 1989	
	Increased external malformations	10	µg/dL	LOAEL	Flora and Tandon 1987	
	Delayed onset of puberty & altered HPG axis in $\mathbb Q$ rats	14	µg/dL	LOAEL	Dearth <i>et al</i> 2002	
	Structural damage to seminiferous tubules and reduced prospermatogonia	14	µg/dL	LOAEL	Corpas <i>et al.</i> 1995	

Table 6. Weight of evidence summary table for reproductive effects of lead.

1. NOAEL: no observed adverse effects level; LOAEL: lowest observed adverse effects level; MRR: mid-range of significant multiple regression model; LRR: minimum range of significant multiple regression model with evidence of non-linearity; IC50: inhibitory concentration 50

2.2.6 Renal Effects

Lead causes proximal tubular injury with a characteristic pathology of proximal tubule nuclear inclusion bodies progressing to tubulointerstitial disease and fibrosis. Lead accumulation in the proximal tubule leads to hyperuricaemia and gout, presumably by inhibiting uric acid secretion, and diminished renal clearance, tubular reabsorption and glomerular filtration rate (GFR) (Gonick, 2008).

Blood lead concentrations greater than 40 µg/dL are associated with increased risk for nephropathy and related renal failure in adults. Lower exposures to lead can act as a cofactor to increase the risk of renal dysfunction and the rate of functional decline. Diabetics and hypertensives are at increased risk of renal dysfunction at lower lead exposures.

GFR is considered the best measure of renal function. Creatinine (serum or urinary) has been most frequently used to measure lead-related renal effects. Unfortunately, this is not a very sensitive measure of GFR or early renal dysfunction. For example, serum creatinine levels are not normally affected by kidney donation.

An inverse association between blood lead and GFR has been reported, after adjusting for confounders, in most environmental cohort studies and this relationship has been observed in cohorts with mean blood lead concentrations as low as 2 μ g/dL (Akesson *et al.*, 2005). A ten-fold increase in blood lead is associated with a decrease in creatinine clearance of 10-15 mL/min (Staessen *et al.*, 1992; Akesson *et al.*, 2005). A 3.5 μ g/dL increase in blood lead concentration has the equivalent adverse impact on GFR as an increase of about five years in age or an increase in body mass index (BMI) of about 7 kg/m². Effect estimates

for susceptible sub-populations, such as those with diabetes, hypertension, or chronic renal insufficiency, are likely to be higher.

Stronger inverse longitudinal relationships between blood lead or bone lead and creatinine clearance have been reported in hypertensives and diabetics (Tsaih *et al.*, 2004). Among hypertensives with a mean blood lead of 4 µg/dL, the adjusted odds ratio for elevated serum creatinine (\geq 99th percentile of population norm) was 1.80 (95% CI: 1.34-2.42) for those with blood lead concentrations of 3.9 to 5.9 µg/dL relative to those with blood lead concentrations of 0.7 to 2.4 µg/dL; an increased adjusted odds ratio of 1.85 (95% CI: 1.32-2.59) for chronic kidney disease (estimated GFR < 60 mL/min) was also reported for the same magnitude of increase in blood lead concentrations (Muntner *et al.*, 2003). Risk factors for renal disease, such as obesity, diabetes, and hypertension, tend to overlap with risk factors for lead exposure in lower SES groups.

Evidence of renal effects in occupational cohorts with generally higher exposures is less consistent, but this literature also generally has greater methodological issues that may be null biasing, such as smaller sample sizes, healthy worker effect, and relatively high lead exposure amongst reference groups. There is also some observational and *in vivo* evidence that renal dysfunction at higher dose exposures may be preceded by hyperfiltration. The data from the few studies on renal effects of lead exposure in children are inconsistent and the significance of the positive results is difficult to interpret because they tended to measure biomarkers of renal dysfunction that are less well understood in terms of their clinical significance.

There are few *in vivo* data on environmentally relevant lead exposures and renal function in animals. Blood lead concentrations in rats of 26 µg/dL produced

increased creatinine clearance and accelerated renal microvascular and tubulointerstitial injury (Roncal *et al.*, 2007). Blood lead concentrations of 29 µg/dL in rats produced transient hyperfiltration – a finding consistent with evidence from relatively high exposure occupational cohort studies. This exposure level also produced mild tubular atrophy and interstitial fibrosis (Khalil-Manesh *et al.*, 1993a).

In summary, reduced GFR, estimated from serum cystatin C, and reduced creatinine clearance has been associated with average blood lead concentrations in environmental cohorts as low as 2.2 μ g/dL (Akesson *et al.*, 2005). These results have been replicated in most, but not all cohort studies and the lowest blood lead concentrations among the corroborating studies is 2.5 to 3.8 μ g/dL, which was a group range for increased risk of chronic kidney disease among hypertensives (Muntner *et al.*, 2003). Based on this evidence, there is suggestive evidence of an effect at blood lead concentrations as low as 2.2 μ g/dL and moderately strong evidence of an effect at blood lead concentrations as low as about 3 μ g/dL. Renal effects have been replicated *in vivo* at blood lead concentrations as low as about 30 μ g/dL; however there are few *in vivo* data on environmentally relevant lead exposures and renal function in animals.

The relevant studies for determining the blood lead weight of evidence heuristic for renal effects are presented in Table 7.
		Blood Pb			
		associated			
		with			
Туре	Outcome	outcome	Units	Metric	Reference
Epi	Reduced GFR and creatinine clearance	2.2	µg/dL	MRR	Akesson <i>et al</i> 2005
	Risk of elevated serum	2.5-3.8	µg/dL	LOAEL	Muntner <i>et al</i> .
	disease amongst hypertensives				2003
	Reduced creatinine clearance	7.5	µg/dL	MRR	Staessen <i>et</i> <i>al</i> . 1992
	Reduced creatinine clearance	8.1	µg/dL	MRR	Payton <i>et al.</i> 1994
In vivo	Reduced creatinine clearance,	26	µg/dL	LOAEL	Roncal <i>et al</i>
	tubulointerstitial injury				2007
	Transient hyperfiltration, tubular	29	µg/dL	LOAEL	Khalil-Manesh
	au opny, and interstitiar infosis				<i>et al</i> , 1993

Table 7. Weight of evidence summary table for renal effects of lead.

1. NOAEL: no observed adverse effects level; LOAEL: lowest observed adverse effects level; MRR: mid-range of significant multiple regression model; LRR: minimum range of significant multiple regression model with evidence of non-linearity; IC50: inhibitory concentration 50

2.2.7 Immunological Effects

Environmental lead exposures do not appear to be cytotoxic to immune cells, rather lead causes dysregulation of the immune system which, in turn, impairs certain host defences and may enhance the risk of allergic and autoimmune disease.

Developmental immunotoxicity is, based on existing experimental evidence, one of the more sensitive of lead's toxic effects. There are a relatively large number of *in vivo* studies and some epidemiological evidence that demonstrate the adverse effects of lead on the immune system at blood lead concentrations less than 10 μ g/dL.

There are four well established characteristic immunotoxic effects associated with lead exposure:

- 1. Skewed relative abundance of Th1 and Th2 T-helper cells towards the Th2 phenotype
- 2. Increased production of IgE and Th2 cytokines (interleukins IL-4, IL-10) and concomitant decrease in Th1 cytokines (IfG and IL-12).
- 3. Depression of Th1 mediated immune response (delayed type hypersensitivity (DTH) response)
- 4. Impaired development and functioning of macrophage populations.

There is experimental and observational evidence of these effects at blood lead concentrations as low as $3-5 \ \mu g/dL$. The epidemiological literature is not entirely consistent, but not all epidemiological studies measured immune responses that are affected by lead. Decreased host resistance has been demonstrated in experimental studies, but there are few observational data on decreased host resistance or other functional effects.

The observed cellular perturbations may result in increased risk of asthma and autoimmune disease, but there are few data on these health consequences. Min *et al.* (2008) reported a significant adjusted association between blood lead concentrations and bronchial responsiveness (as assessed with methacholine challenge) among adults with average blood lead concentrations of 2.9 μ g/dL. A single study reports no association between blood lead concentrations of about 5 μ g/dL in children and asthma (Joseph *et al.*, 2005).

There is observational and experimental evidence that blood lead concentrations less than 10 μ g/dL result in elevated plasma IgE and other perturbations in the relative abundance of circulating cytokines and immunoglobulin (Sarasua *et al.*,

2000; Snyder *et al.*, 2000; Sun *et al.*, 2003; Karmaus *et al.*, 2005). Elevated IgE has been associated with increasing blood lead concentrations in children with average bood lead concentrations as low as about 3 μ g/dL (Karmaus *et al.*, 2005) and animals with blood lead concentrations as low as 5 μ g/dL (Snyder *et al.*, 2000). Excessive IL-4 (Th2 cytokine) results in an increase in IgE; elevated IgE may increase the risk of IgE mediated atopy and asthma.

There is experimental evidence that blood lead concentrations less than 10 μ g/dL can impair the development of macrophage populations and alter the function of matured macrophages (Bunn *et al.*, 2001). The effect has also been reported in children with blood lead concentrations of about 10 μ g/dL (Pineda-Zavaleta *et al.*, 2004). Lead-affected macrophages display depressed NO production and increased production of pro-inflammatory cytokines and reactive oxygen specides (ROS: superoxide anion and hydrogen peroxide). NO production by macrophages is critical to immune defence against infection. It is noted that the production of ROS and the subsequent depression of NO are also key events in one of the putative modes of action for the hypertensive effects of lead.

The developing fetus, neonate, and infants are more susceptible to lead-induced immunotoxic effects. There is a three to twelve fold difference in reported *in vivo* lowest observed adverse effects levels (LOAELs) between perinatal and adult lead exposure periods for various immunotoxic effects (Dietert and Piepenbrink, 2006). Relatively short (subchronic) periods of early-life lead exposure can result in latent immune dysfunction . Stress or dietary deficiencies may modify the developmental immunotoxicity of lead.

Summary

Lead alters the balance of immune cell types and associated cytokines and immunoglobulins. Elevated IgE has been associated with increasing blood lead concentrations in children with average blood lead concentrations as low as about 3 µg/dL (Karmaus et al., 2005) and animals with blood lead concentrations as low as 5 µg/dL (Snyder et al., 2000). Disruption of the relative balance of immune cell types results in altered immune function; impaired delayed type hypersensitivity (DTH) response is induced *in vivo* with blood lead concentrations as low as 7 µg/dL (Chen et al., 2004). Higher blood lead concentrations in vivo have been associated with decreased host resistance to viruses, bacteria, and malignant tumours. The health consequences of lead-induced dysregulation of the immune system have not been well studied in humans, but may include increased risk for atopy, asthma and autoimmune disease. On this basis, there is suggestive evidence of effects associated with blood lead concentrations as low as 3 µg/dL, moderate evidence of effects at blood lead concentrations as low as 5 µg/dL, and strong evidence of effects at blood lead concentrations as low as 7 µg/dL.

The relevant studies for determining the blood lead weight of evidence heuristic for immunological effects are presented in Table 8.

		Blood Pb associated			
		with			
Туре	Outcome	outcome	Units	Metric	Reference
Epi	Increased serum IgE in children	3	µg/dL	MRR	Karmaus <i>et al</i> 2005
	Increased serum IgA, IgG & IgM in	7	µg/dL	MRR	Sarasua <i>et al</i>
	children				2000
	Increased bronchial responsiveness	3	µg/dL	MRR	Min <i>et al</i> 2008
	No relationship with asthma in children	5	µg/dL	MRR	Joseph <i>et al</i> 2005
In vivo	Increased serum IgE in mouse	5	µg/dL	LOAEL	Snyder <i>et al</i>
					2000
	Reduced delayed type hypersensitivity response in rats	7	µg/dL	LOAEL	Chen <i>et al</i> 2004
	Decreased circulating macrophages in rats	8.2	µg/dL	LOAEL	Bunn <i>et al</i> 2001

Table 8. Weight of evidence summary table for immunological effects of lead.

1. NOAEL: no observed adverse effects level; LOAEL: lowest observed adverse effects level; MRR: mid-range of significant multiple regression model; LRR: minimum range of significant multiple regression model with evidence of non-linearity; IC50: inhibitory concentration 50

2.2.8 Effects on Bone and Teeth

Lead may affect bone growth and density through direct toxic effects or indirectly through effects on endocrine function. There are few observational data on the effects of lead on bone health and the experimental data are dervied from studies which employed relatively high exposures (e.g., blood lead concentraitons > 40 μ g/dL).

In vivo blood lead concentrations as low as 17 μ g/dL have been shown to lower bone density in rats (Gruber *et al.*, 1997). Depressed rates of early bone growth were observed in rats with blood lead concentrations of about 50 μ g/dL but not at

blood lead concentrations of 43 μ g/dL (Camoratto *et al.*, 1993). Campbell *et al.* (2004) reported that children with high lead exposure (geomean 23.6 μ g/dL) had higher bone mineral density (BMD) than children with low blood lead concentrations. The authors hypothesize that these findings may be due to an accelerated maturation of bone.

The observational data on the effects of lead on stature and growth are suggestive of an effect, but these studies have generally not done a good job controlling for confounders, such as nutrition.

Lead exposure may promote dental caries. The epidemiological evidence at low exposures is inconsistent, however, and the few experimental data for this outcome are from relatively high exposures. Moss *et al.* (1999) and Gemmel *et al.* (2002) report an association between blood lead and risk of dental caries in subjects (children and adults) with average blood lead concentrations about 2-3 μ g/dL; however, Campbell *et al.* (2000) reported that there was no increased risk of dental caries in children with blood lead concentrations greater than 10 μ g/dL. Dye *et al.* (2002) reports that blood lead was associated with periodontal bone loss among ever smokers or current smokers with average blood lead concentrations of 2.5 μ g/dL. Watson *et al.* (1997) reported that blood lead concentrations in rats of 48 μ g/dL increased the incidence of dental caries in rat pups by approximately 40%.

In summary, there is inconsistent epidemiological evidence of adverse effects on oral health at environmental blood lead concentrations as low as 2 μ g/dL. Blood lead concentrations as low as 17 μ g/dL have been shown to cause adverse effects on bone health in experimental animals, but the *in vivo* evidence for

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adverse effects on bone health is not corroborated until blood lead concentrations reach about 50 µg/dL.

The relevant studies for determining the blood lead weight of evidence heuristic for effects on bone and teeth are presented in Table 9.

	Blood Pb					
		associated				
		with				
Туре	Endpoint	outcome	Units	Metric ¹	Reference	
Epi	Increased risk of dental caries	2.1	µg/dL	MRR	Moss <i>et al</i> 1999	
	Increased periodontal bone loss among smokers	2.5	µg/dL	MRR	Dye <i>et al</i> 2002	
	Increased risk of dental caries among rural children	2.9	µg/dL	LOAEL	Gemmel <i>et al</i> 2000a	
	Increased risk of dental caries in deciduous teeth (<i>p=0.07</i>)	10	µg/dL	LOAEL	Campbell <i>et al</i> 2000a	
	Increased BMD in children	23.6	µg/dL	LOAEL	Campbell <i>et al</i> 2004	
	No association with risk of dental caries among urban children	1.7	µg/dL	NOAEL	Gemmel <i>et al</i> 2000a	
	No association with risk of dental caries in permanent teeth	10	µg/dL	NOEAL	Campbell <i>et al</i> 2000a	
In vivo	Decreased bone density in rats	17	µg/dL	LOAEL	Gruber <i>et al</i> 1997	
	No effect on bone growth in rats	43.3	µg/dL	NOAEL	Camoratto <i>et al</i> 1993	
	Increased dental caries in rats	48	µg/dL	LOAEL	Watson <i>et al</i> 1997	

Table 9. Weight of evidence summary table for effects of lead on teeth and bone.

1. NOAEL: no observed adverse effects level; LOAEL: lowest observed adverse effects level; MRR: mid-range of significant multiple regression model; LRR: minimum range of significant multiple regression model with evidence of non-linearity; IC50: inhibitory concentration 50

2.2.9 Cancer

Inorganic lead is classified by IARC as a 2B carcinogen. Other health agencies, including Health Canada (this report) have reached similar conclusions. This classification is based on sufficient evidence in experimental studies but inconsistent observational evidence. The epidemiological studies of an association between lead exposure and cancer have primarily been done on occupational cohorts and most did not sufficiently control for potential confounders. The few observational studies of environmental exposures are equivocal, but there are no longitudinal studies with serial lead exposure measures. Lead is a confirmed animal carcinogen, but experimental exposures, in contrast to most other endpoints, have been at very high lead doses. Experimental evidence indicates that perinatal lead exposure may be more potent than adult exposure. Lead appears to be genotoxic, but it is not clear if this is through direct or indirect means. Lead does not appear to be a mutagen.

The only environmental cohort studies relating blood lead concentrations to cancer are follow-up studies from NHANES II (1976-1980) and NHANES III (Schober, 2006). The findings from NHANES II, where the mean blood lead concentration was 14 µg/dL were equivocal (Jemal, 2002; Lustberg and Silbergeld, 2002). The findings from NHANES III are also not entirely consistent, with Schober *et al.* (2006) reporting an increased relative risk for cancer mortality for subjects with a blood lead of 5-10 µg/dL at baseline and Menke *et al.* (2006) reporting no significant association with blood lead concentrations. One explanation for the difference in results between these two similar studies is that Schober *et al.* (2006) excluded subjects less than 40 years of age while Menke *et al.* (2006) included all subjects over 20 years of age; therefore the older study population of Schober *et al.* (2006) may be more sensitive. A population case control study did not report any association between risk of breast cancer and urinary lead (McElroy *et al.*, 2008).

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The lowest oral exposures in animal assays that produced renal tumours were 500 ppm lead acetate in drinking water of dams (perinatal exposure) (Waalkes *et al.*, 1995) and 1,000 ppm lead acetate in drinking water of male mice in an adult lifetime exposure study (Waalkes *et al.*, 2004). The blood lead concentrations in these animals were not reported. Based on blood lead concentrations achieved in other mouse studies with similar exposure magnitude (100 mg/kg/d or 200 mg/kg/d), route, duration and timing, it is estimated that these animals had blood lead concentrations of about 50 µg/dL and 100 µg/dL, respectively.

In summary, there is equivocal epidemiological evidence of an association between blood lead concentrations as low as 5-10 μ g/dL and cancer. Lead causes renal tumours in rodents at exposures that are estimated to produce blood lead concentrations of 50-100 μ g/dL.

The relevant studies for determining the blood lead weight of evidence heuristic for carcinogenic effects are presented in Table 10.

		Blood Pb			
		with			
Туре	Outcome	outcome	Units	Metric	Reference
Epi	Non-significant increase in cancer mortality for subjects > 20 yrs	>3.63	µg/dL	NOAEL	Menke <i>et al</i> 2006
	Increased risk of cancer mortality for subjects > 40 yrs	5-9	µg/dL	LOAEL	Schober <i>et al</i> 2006
	No increased risk of cancer mortality among Caucasians	14	µg/dL	MRR	Jemal <i>et al</i> 2002
	Increased risk of cancer mortality relative to subjects with blood lead < 19 μg/dL	> 20	µg/dL	LOAEL	Lustberg & Selbergeld 2002
In vivo	Increased incidence of renal proliferative lesions in mice (perinatal exposure only)	50*	µg/dL	LOAEL	Waalkes <i>et al</i> 2004
	Increased incidence of renal proliferative lesions in mice (adult exposure only)	100*	µg/dL	LOAEL	Waalkes <i>et al</i> 1995

Table 10. Weight of evidence summary table for carcinogenic effects of lead.

1. NOAEL: no observed adverse effects level; LOAEL: lowest observed adverse effects level; MRR: mid-range of significant multiple regression model; LRR: minimum range of significant multiple regression model with evidence of non-linearity; IC50: inhibitory concentration 50

*Blood Pb data not reported for these studies; estimated blood Pb based on blood Pb achieved in other mouse studies with similar exposure magnitude (100 mg/kg/d or 200 mg/kg/d), route, duration and timing.

2.2.10 Effects to the Ocular and Visual System

The most frequently reported sensory system alterations occur in the visual system. While there is evidence to indicate that environmental lead exposure can have adverse effects on auditory function, the focus here is on effects on the ocular and visual system under the assumption that it will be more sensitive to low-level effects. it is noted that lead-induced auditory and visual deficits can adversely affect learning and memory as well as experimental procedures used to assess cognitive function.

It is well established that higher lead exposures have toxic effects on the ocular and visual systems. More recent experimental and epidemiological evidence shows that lead can have adverse effects at lower doses and that the nature of these effects is dependent on dose and timing of exposure. In rats, relatively high lead exposure has adverse effects on both the retina and the optic nerve whereas lower exposures primarily affect rod photoreceptors and their associated bipolar cells (Fox and Boyes, 2008).

Overt lead poisoning (i.e. blood lead concentrations > 80 µg/dL) can cause visual symptoms and visual system pathology. Moderate blood lead levels in occupational cohorts and developmentally exposed experimental animals produce scotopic and temporal visual system deficits. Gestational lead exposure in an environmentally exposed cohort was associated with supernormal scotopic electroretinograms (ERGs) in 7-10 year old children. There are no other data on environmentally exposed cohorts.

Low gestational lead exposure (12 µg/dL) in rats produced supernormal ERGs increased neurogenesis of rods, and decreased retinal dopamine (DA). High lead exposure (46 µg/dL) produced further loss of DA, rod cell loss and ERG subnormality. These deficits are associated with an increased risk of spatiotemporal contrast sensitivity deficits.

A case report describes a 10 year old girl with a peak measured blood lead concentration of 19 μ g/dL who had supranormal scotopic and photopic ERGs and clinical findings of decreased visual acuity, color vision, and stereopsis upon follow-up examination five years after the peak lead exposure (Nagpal and Brodie, 2009).

The relevant studies for determining the blood lead weight of evidence heuristic for effects on the ocular and visual systems are presented in Table 11.

		Blood Pb			
		associated			
		with			
Туре	Outcome	outcome	Units	Metric	Reference
Epi	Supernormal scotopic ERG in children	10.5	µg/dL	LOAEL	Rothenberg <i>et al</i> 2002b
ln vivo	Supernormal scotopic ERG, neurogenesis of rods and bipolar cells, decreased retinal DA	12	µg/dL	LOAEL	Fox <i>et al</i> 2008
	Scotopic ERG abnormalities in rats	19	µg/dL	LOAEL	Fox <i>et al</i> 1991
	Loss of rod and bipolar cells in rats, delayed dark adaption,	20	µg/dL	LOAEL	Fox <i>et al</i> 1997
	Apoptosis of rod cells in rats	26	µg/dL	LOAEL	He <i>et al</i> 2003
	Subnormal scotopic ERG, loss of rods and bipolar cells	46	µg/dL	LOAEL	Fox <i>et al</i> 2008

1. NOAEL: no observed adverse effects level; LOAEL: lowest observed adverse effects level; MRR: mid-range of significant multiple regression model with evidence of non-linearity; IC50: inhibitory concentration 50

2.2.11 Effects on the Endocrine System

Endocrine effects related to specific systems are discussed in their respective sections above. Effects on adrenal hormones (glucocorticoids and catecholamines), thyroid, growth, and calcitropic endocrine systems are discussed here. There is strong experimental evidence that lead perturbs adrenal hormones at blood lead concentrations as low as 20-40 µg/dL. The evidence for other endocrine effects is inconsistent or negative. There are few data on endocrine effects at environmentally relevant blood lead concentrations.

There is strong experimental evidence that lead exposure can interfere with the functioning of the hypothalamic-pituitary-adrenal (HPA) axis. Activation of the

HPA axis plays a pivotal role in the stress response. Short-term activation of the HPA axis allows adaptive responses to a challenge; however, chronic dysfunction of the HPA axis can have detrimental effects on an organism: HPA dysfunction has been implicated in the etiology of sleep disturbances, anxiety, depression, obesity, hypertension, diabetes, and schizophrenia. Increased levels of glucocorticoids can also depress cell mediated immune responses. *In utero* exposure to elevated glucocorticoids has been shown in a variety of animal models to alter the adult physiology of the offspring and produce permanent hyperglycemia, hypertension, hyper-responsive HPA axis, and behavioural effects that suggest heightened anxiety (Seckl and Meaney, 2004; Darnaudery and Maccari, 2008).

Blood lead concentrations as low as 30-40 µg/dL have been shown to alter HPA axis functioning in test animals. Maternal stress and perinatal lead exposure have been shown independently to alter the function of the HPA axis. Research by Dr. Deborah Cory-Slechta and colleagues has shown that maternal lead and stress can have an interactive effect on the functioning of the HPA axis in adult offspring in a rodent model. The pattern of effects is complex and appears to be dependent on dose, timing, sex and endpoint, but in general perinatal lead exposure results in increased levels of glucocorticoids (corticosteroids) in offspring and lead potentiates the effects of maternal stress in female offspring (Cory-Slechta *et al.*, 2004; Cory-Slechta *et al.*, 2008; Rossi-George *et al.*, 2009).

Lead exposure *in vivo* producing blood lead concentrations of 20-30 μ g/dL resulted in increased plasma catecholamine (plasma norepinepherine and adrenaline) and altered tissue monoaminooxidase (MAO) and β -adrenergic receptor levels (Chang *et al.*, 1997; Carmignani *et al.*, 2000).

Blood lead concentrations were associated with elevated plasma catecholamine levels, decreased β_2 -adrenergic receptors, and elevated blood pressure in lead-exposed workers with average blood lead concentrations of 49 µg/dL (Chang *et al.*, 1996). No observational studies of the effects of lead exposure on glucocorticoids were identified at the time of this report.

Lead exposure can alter plasma levels of vitamin D metabolites [25-OHD3 and $1,25-(OH)_2D_3$] – the direction of effect, however, may be dose dependent and mediated by dietary calcium. The epidemiological evidence of an association between blood lead concentrations and calcitropic endocrine function are inconsistent. No effects on serum $1,25-(OH)_2D_3$ and blood lead concentrations were observed in calcium replete children with a mean lifetime average blood lead concentration of about 10 µg/dL (Koo *et al.*, 1991), but higher blood lead concentrations have been associated with depressed $1,25-(OH)_2D_3$. *In vivo* effects have only been reported at relatively high blood lead concentrations (> 50 µg/dL) or in association with relatively high oral doses.

Basal and stimulated growth hormone release in rats was not affected by perinatal lead exposure producing blood lead concentrations of 43 µg/dL (Camoratto *et al.*, 1993).

Most of the epidemiological evidence of effects on thyroid endocrine function is from occupational cohorts. The findings are inconsistent. The inconsistency among findings may be because the direction of effect is dependent on the magnitude or duration of exposure. Hyperparathyroidism has been observed in association with hypocalcemia and decreased $1,25-(OH)_2D_3$ in rats with blood lead concentrations greater than 40-50 µg/dL. A group of male adolescent auto mechanics with higher than normal blood lead concentrations (mean = 7 µg/dL) had higher levels of free thyroxine (FT4) than controls; no difference in free triiodothyronine (FT3), thyrotrophin (TSH), or thyroid volume was reported (Dundar *et al.*, 2006). Occupational studies with higher blood lead concentrations have reported no effects on thyroid hormones, however. The relevant studies for determining the blood lead weight of evidence heuristic for endocrine effects are presented in Table 12.

		Blood Pb			
		associated			
		with			
Туре	Outcome	outcome	Units	Metric	Reference
Epi	Increased FT4, but no change in FT3, TSH, or thyroid volume	7	µg/dL	LOAEL	Dundar <i>et al</i> 2006
	Increased T3 & T4	17	µg/dL	MRR	Dursun and Tutus (1999)
	No association with serum vitamin D in children	10	µg/dL	NOAEL	Koo <i>et al</i> 1991
	No change in T3	25	µg/dL	MRR	Siegel <i>et al.</i> (1989)
	No change in T3, T4, or TSH	24	µg/dL	MRR	Schumacher <i>et</i> <i>al.</i> (1998)
In vivo	Increased corticostreiod levels in rats following perinatal Pb exposure; maternal stress potentiated effects in female offspring	30-40	µg/dL	LOAEL	Cory-Slechta et al. 2004
	Increased plasma catacholimines and altered tissue β -adrenergic receptor densities	30	µg/dL	LOAEL	Chang <i>et al</i> 1997
	Increased plasma norepinepherine and monoamineoxidase levels	28	µg/dL	LOAEL	Carmignani <i>et al</i> 2000
	No significant effect on basal or stimulated release of growth hormone from pituitary gland	43	µg/dL	LOAEL	Camoratto <i>et al</i> 1993

Table 12. Weight of evidence summary table for effects of lead on the endocrine systems.

1. NOAEL: no observed adverse effects level; LOAEL: lowest observed adverse effects level; MRR: mid-range of significant multiple regression model; LRR: minimum range of significant multiple regression model with evidence of non-linearity; IC50: inhibitory concentration 50

2.3 BLOOD LEAD WEIGHT OF EVIDENCE HEURISTIC

The data summary tables for each toxicological summary narrative (see above) were used to generate the blood lead weight of evidence heuristic model, Figure 2. The blood lead weight of evidence heuristic model provides a snap-shot of the relative weight of evidence for each of the major organs or systems affected by lead.



Figure 2. Blood lead weight of evidence heuristic model for the toxicological effects of lead

2.3.1 Discussion

Of the organ systems examined in this report, there were none with consistent evidence of no effects below a certain blood lead concentration. There is, to varying degrees, some evidence of adverse effects on all organ systems down to the lowest blood lead concentrations studied.

The following systems have at least moderately strong evidence of adverse effects associated with chronic blood lead concentrations < 10 μ g/dL:

- Neurological
- Cardiovascular
- Reproductive
- Haematopoietic
- Immune
- Renal

Moderately strong evidence of adverse effects is defined as: (1) multiple epidemiological studies that are predominantly positive for an effect within this range, or (2) a single animal study that is positive for an effect within this range.

The lowest blood lead concentration at which a particular category of weight of evidence is satisfied is a product of:

 How intensely an endpoint has been studied. A lack of studies can limit the weight of evidence that can be achieved. Alternatively, a large number of studies on an endpoint, such as IQ, increases the likelihood that there will be some inconsistency among the results.

- The sensitivity and specificity of the endpoint studied. For example, an endpoint like decreased blood ALAD is both very sensitive and very specific for the haematopoietic effects of lead. In contrast, some measures, like IQ as a measure of neurological injury or urinary creatinine as a measure of GFR, are neither specific nor sensitive. The likelihood of inconsistent results increases with relatively global and insensitive measures of effect.
- The degree to which investigators have attempted to define the shape of the dose-response curve. Endpoints, like IQ, where there is evidence that the dose-response relationship extends to the lowest ranges of study data can appear more "sensitive" than endpoints where effects have simply been shown across a broad range of data.

It is important to keep these factors in mind, particularly in the context of trying to define the most "sensitive" endpoints.

It is emphasized that this is a heuristic model and it was not intended to support fine distinctions and detailed inferences. Rather, it is the overall pattern and trends that are important. Some of these trends are discussed below.

No Threshold

For several health outcomes, including effects on IQ, blood pressure, and delay to onset of puberty, adverse effects have been reported at the lowest exposures studied in both epidemiological and experimental studies. Therefore, there are several endpoints for which a threshold for the adverse effects of has yet to be identified.

Common Modes

The wide variety of tissues and systems adversely affected by lead and the complexity of lead toxicity may be explained by the presence of some common underlying modes of toxicity that have the potential to affect the functioning of many different cell types. These potential mechanisms include oxidative and nitrosative stress, Ca²⁺ and Zn²⁺ mimicry, and sulphydryl (thiol group) binding. Some of these modes of toxicity are also common to other metals, such as mercury and cadmium.

Some of the modes of toxicity associated with lead, such as oxidative and nitrosative stress and altered DNA methylation and associated gene expression, also contribute to the pathogenesis of diseases with multiple environmental and hereditary risk factors, such as atherosclerosis, hypertension, neurodegenerative disease, renal dysfunction, and cancer. In this context, the adverse effects associated with environmental lead exposures can be thought of as an additional risk factor that can exacerbate and accelerate the onset of these diseases.

Oxidative stress is implicated in the pathogenesis of hypertension, atherosclerosis, neurodegenerative disease, cancer, renal dysfunction and other diseases. Lead exposure results in increased tissue concentrations of reactive oxygen and reactive nitrogen species, including hydrogen peroxide, peroxynitrite, and hydroxyl. There is evidence that lead-induced oxidative and nitrosative stress is a plausible mechanism for the haematopoietic, cardiovascular, male reproductive, renal, and neurological consequences of lead exposure.

Ca²⁺ mimicry can disrupt cellular signalling, reduce cellular energy, and disrupt neurotransmission. Disruptions in cellular signalling can result in apoptosis and altered gene expression. Zn²⁺ mimicry can result in altered protein function. One of the best known adverse effects of lead is its impairment of the Zn²⁺ containing proteins delta-aminoluvulinic acid dehydratase and ferrochelatase and the resulting

impairment of haeme synthesis. There are over 300 other Zn²⁺ containing enzymes and over 2,000 Zn-dependent transcription factors in the human body that could also be targets for Pb²⁺ substitution. Lead also irreversibly binds to sulphydryl (or thiol) groups of proteins and causes protein dysfunction.

More detailed and specific descriptions of the particular mechanisms of lead toxicity often include one of these common modes. The ability of lead to result in modes of toxicity that can adversely affect many cell types and physiological functions are consistent with the findings that lead can adversely affect a wide variety of tissues and systems.

2.4 CRITICAL EFFECTS

TRVs are derived on the basis of a quantified dose-response relationship for a single health endpoint. Traditionally, this approach has been justified by ensuring that the TRV is derived on the basis of the "critical", or most sensitive, effect. However, in the case of lead there is insufficient evidence to identify any single effect as critical by the strict definition of the term. As an alternative, for the purpose of this report, the critical effects of lead were defined as those endpoints for which the blood lead concentration-response relationship was relatively well described in the literature.

Blood lead concentration-response relationships have been or could likely be quantified for any of the six systems for which at least moderately strong evidence of adverse effects were found at blood lead concentrations less than 10 µg/dL (neurological, reproductive, cardiovascular, haematopoietic, immune, and renal). However, the endpoints for which blood lead concentration-response relationships have been most commonly published and analyzed are:

• Early life blood lead concentrations and deficits in IQ among school-aged children, and

• Adult blood lead concentrations and increased systolic blood pressure (SBP).

On this basis these endpoints were identified as "critical" effects for deriving TRVs for environmental lead exposure.

It is emphasized that these are not the only endpoints for which there is evidence of adverse effects associated with relatively low blood lead concentrations. Risk assessors should keep in mind that these endpoints, to a degree, are surrogates for all of the other potential health effects associated with a given blood lead concentration.

Blood lead and hypertension risks are the endpoints that have been most commonly related to bone lead concentrations. In an earlier draft of this report a TRV for lead had been developed on the basis of the relationship between tibia lead and SBP. While the literature allows for the development of a quantitative estimate of the relationship between tibia lead and SBP, there is greater uncertainty in this relationship than that for blood lead and SBP. Also, the slope of the tibia lead-SBP relationship did not appear to be consistent with that developed for blood lead-SBP. Reconciling these differences was difficult because of the uncertainty in the relationship between cumulative blood lead index (CBLI) and tibia lead concentrations, particularly at relatively low environmental lead exposures. Therefore, it was decided not to derive a TRV on the basis of tibia lead and SBP in the final version of this report.

2.5 CANCER

The weight of evidence for carcinogenic effects of lead at low exposure doses is relatively weak. However, in 2004 IARC re-classified inorganic lead compounds as Group 2A Carcinogens (probably carcinogenic to humans) (IARC, 2006). It is Health

Canada policy to derive quantitative estimates of carcinogenic potency for substances classified under the Canadian Environmental Protection Act (CEPA) classification scheme as Group I or II Carcinogens (Health Canada, 1994; Health Canada, 1995; Health Canada, 1996). The criteria for a CEPA Group II Carcinogen are very similar to the criteria for an IARC 2A carcinogen. Therefore, cancer was carried forward for evaluation under the CEPA classification scheme for carcinogens. The evaluation and cancer slope factors are presented in Section 7 of the report.

2.6 SUMMARY AND CONCLUSIONS

The purpose of this section was to provide an overall summary of the evidence of the toxic effects of lead, as measured by blood lead concentrations, on various organs and systems. A secondary objective was to identify the "critical effects" that will be carried forward for derivation of Toxicological Reference Values (TRVs). Information on the potential health effects of lead exposure is expressed in terms of blood lead concentrations because this is the exposure metric that is most commonly used in both experimental and observational studies of the toxic effects of lead.

Data were abstracted from original papers or review articles to identify the lowest blood lead concentration associated with an effect or the highest lowest blood lead concentration where there was no effect. Summary information on the toxic effects of lead was presented in two formats: Narrative toxicological summaries were developed for each of the major organ systems adversely affected by lead. Data from the narrative summaries were integrated into a blood lead weight of evidence heuristic model. This heuristic model provides a graphical representation of the weight of evidence for effects on different systems at various blood lead concentrations. Of the organ systems examined, there were none with consistent evidence of no effects below a certain blood lead concentration. There is, to varying degrees, some evidence of adverse effects on all organ systems down to the lowest blood lead concentrations studied.

The wide variety of tissues and systems adversely affected by lead and the complexity of lead toxicity may be explained by the presence of some common underlying modes of toxicity that have the potential to effect the functioning of many different cell types. These potential mechanisms include oxidative and nitrosative stress, Ca²⁺ and Zn²⁺ mimicry, and sulphydryl (thiol group) binding. Some of these modes of toxicity are also common to other metals, such as mercury and cadmium.

Some of the modes of toxicity associated with lead, such as oxidative and nitrosative stress and altered DNA methylation and associated gene expression, also contribute to the pathogenesis of diseases with multiple environmental and hereditary risk factors, such as atherosclerosis, hypertension, neurodegenerative disease, renal dysfunction, and cancer. In this context, the adverse effects associated with environmental lead exposures can be thought of as an additional risk factor that can exacerbate and accelerate the onset of these diseases.

The following systems have at least moderately strong evidence of adverse effects associated with chronic blood lead concentrations < 10 μ g/dL:

- Neurological
- Cardiovascular
- Reproductive
- Haematopoietic
- Immune
- Renal

Moderately strong evidence of adverse effects is defined as (1) multiple epidemiological studies that are predominantly positive for an effect within this range; or (2) a single animal study that is positive for an effect within this range.

TRVs are derived on the basis of a quantified dose-response relationship for a single health endpoint. Traditionally, this approach has been justified by ensuring that the TRV is derived on the basis of the "critical", or most sensitive, effect. However, in the case of lead there is insufficient evidence to identify any single effect as critical by the strict definition of the term. To do so would require an understanding of the relative thresholds for the various adverse effects of lead – something that is currently absent.

As an alternative, for the purpose of this report, the critical effects of lead were defined as those endpoints for which the blood lead concentration-response relationship was relatively well described in the literature. These endpoints are:

- Early life blood lead concentrations and deficits in IQ and
- Adult blood lead concentrations and increased systolic blood pressure (SBP).

The toxicological evidence for adverse effects on IQ and SBP is reviewed in detail in Sections 3 and 4, of this report, respectively.

Blood lead concentration-response relationships are quantified for these endpoints in Section 5 of the report.

Finally, risk-specific blood lead concentrations for population level health effects on IQ and SBP are presented in Section 6 of the report.

It is emphasized that these are not the only endpoints for which there is evidence of adverse effects associated with relatively low blood lead concentrations. Risk assessors should keep in mind that these endpoints, to a degree, are surrogates for all of the other potential health effects associated with a given blood lead concentration.

A TRV based on tibia lead and SBP was not derived because: (1) this relationship has not been as well studied and is associated with greater uncertainty than the relationship between blood lead and SBP; and (2) uncertainty in the relationship between cumulative blood lead index and tibia lead concentrations make it difficult to ensure that TRVs based on blood lead and tibia lead are internally consistent.

The weight of evidence for carcinogenic effects of lead at low exposure doses is relatively weak. However, in 2004 IARC re-classified inorganic lead compounds as Group 2A Carcinogens (probably carcinogenic to humans) (IARC, 2006). It is Health Canada policy to derive quantitative estimates of carcinogenic potency for substances classified under the Canadian Environmental Protection Act (CEPA) classification scheme as Group I or II Carcinogens (Health Canada, 1994; Health Canada, 1995; Health Canada, 1996). The criteria for a CEPA Group II Carcinogen are very similar to the criteria for an IARC 2A carcinogen. Therefore, cancer was carried forward for evaluation under the CEPA classification scheme for carcinogens. The evaluation and cancer slope factors are presented in Section 7 of this report.

SECTION 3 • NEURODEVELOPMENTAL TOXICITY

3.1 INTRODUCTION

There are multiple lines of evidence, including human epidemiological studies, *in vivo* animal assays, and *in vitro* experiments, that support the hypothesis that chronic lead exposure results in developmental neurotoxicity in humans. This report does not provide a comprehensive review and analysis of all of the literature on this subject because recent, comprehensive summaries of the evidence have been presented elsewhere (IARC, 2006; US EPA, 2006; US ATSDR, 2007). All of these reviews support the conclusion that there is sufficient evidence to support a causal relationship between lead exposure and developmental neurotoxicity in humans. The intent of this report is to present adequate evidence to support Health Canada's decisions in the derivation of the toxicological references values for lead.

Lead exposure has been associated with adverse developmental effects on a variety neurological endpoints including:

- Neuromotor function (Dietrich *et al.*, 1993a; Wasserman *et al.*, 2000b; Ris *et al.*, 2004; Despres *et al.*, 2005; Fraser *et al.*, 2006)
- Academic achievement (Needleman *et al.*, 1990; Fergusson *et al.*, 1997; Al-Saleh *et al.*, 2001; Wang *et al.*, 2002; Miranda *et al.*, 2006)
- Delinquent or antisocial behaviour (Fergusson *et al.*, 1993; Bellinger *et al.*, 1994b; Needleman *et al.*, 1996; Dietrich *et al.*, 2001; Needleman *et al.*, 2002; Wright *et al.*, 2008)
- Attention and executive function (Bellinger *et al.*, 1994a; Canfield *et al.*, 2003b; Chiodo *et al.*, 2004; Ris *et al.*, 2004; Braun *et al.*, 2006; Chiodo *et al.*, 2007)

- Auditory function (Schwartz and Otto, 1991; Dietrich *et al.*, 1992; Osman *et al.*, 1999) and
- Visual function (Fox *et al.*, 1997; Fox *et al.*, 2008; Laughlin *et al.*, 2008; Rothenberg *et al.*, 2002b)

However, the endpoint that has been most studied and for which there is the greatest weight of evidence of a causal relationship is the adverse consequences of early life lead exposure on psychometric tests of intelligence (IQ) among school-aged children. Population level risk of mild mental retardation (MMR) was modeled as a function of IQ to help define an appropriate Benchmark Response (BMR) decrement in IQ to derive a toxicological reference value for lead (see Appendix A).

There is also evidence of neurotoxicity of lead in adults, particularly the elderly. However, as a whole, neurological endpoints have been considerably less studied in adults and the weight of evidence and the quantification of biomarker-response relationships are not mature enough to support the derivation of a TRV explicitly on the basis of lead's potentially neurotoxic effects on adults. The adult lead TRV that has been developed based on the vascular effects of lead in adults is also expected to afford an unquantified degree of protection against potential neurological effects of lead on adults.

This section of the report will:

Review the relevant evidence from epidemiological studies on the association between biomarkers of early life chronic lead exposure and developmental neurotoxicity, with an explicit emphasis on the association between children's blood lead and deficits in IQ. The epidemiological evidence is strongly suggestive, but not entirely consistent, of an association between early life chronic lead exposure (as measured by various biomarkers) and decrements in school-aged children's IQ. There is evidence that the developmental neurotoxic effects associated with childhood blood lead concentrations persist out to at least the late teen-age years. Several studies provide evidence of a lack of threshold down to the lowest blood lead concentrations measured in their studies – in the range of 1-2 μ g/dL. There is also evidence of and plausible biological explanations for a steeper biomarker-response relationship at relatively low blood lead concentrations.

Review the available evidence from in vivo experiments on the association between chronic early life lead exposure in laboratory animals and developmental neurotoxicity. Chronic oral exposure to lead in laboratory animals impairs performance on behavioural tests of cognitive function. These effects have been demonstrated in multiple species including at least 2 species of non-human primate. Statistically significant impairment on behavioural tests of cognitive function have been reported in animals with average concurrent blood lead concentrations as low as approximately10 µg/dL. Neurobehavioural effects in animals have been shown to persist after cessation of lead exposure and blood lead concentrations have returned to normal. No threshold for lead induced behavioural deficits in laboratory animals has been established.

Review the available evidence on plausible mechanisms of action by which chronic early life lead exposure could result in developmental neurotoxicity. Lead has been shown to interact with all cell types in the central nervous system. Several mechanisms of neurotoxicity have been demonstrated *in vitro* and *in vivo*. Cellular effects have been demonstrated *in vitro* at Pb²⁺ exposure concentrations as low as the picomolar range. The equivalent *in vivo* whole blood lead concentrations are uncertain, but are likely within the range of those produced by contemporary environmental lead exposures in Canada and possibly 100 times lower.

Review the available evidence on variability in response. There is evidence that environmental stress and genetic polymorphisms may modify the relationship between lead exposure and neurotoxicity.

Justify, on the basis of the most certain biomarker-response relationship, the identification of candidate biomarker-endpoint combinations for TRV development. The endpoint for which the blood lead concentration-response has been most often investigated and which can be characterized with the greatest certainty is the relationship between post natal blood lead concentration and IQ decrements in school-aged children. The biomarker-response relationships for other biomarkers, such as maternal lead, bone lead, and dentine lead, and other neurodevelopmental endpoints, such as behaviour or academic achievement, are relatively uncertain. Therefore, it is recommended that TRVs.not be derived explicitly for these alternate biomarker-endpoint relationships. However, there is no reason to believe that the fetus is any less sensitive to the neurotoxic effects of lead as a school-aged child and maternal blood lead and fetal blood lead concentrations are well correlated. Therefore, the TRV based on the effect of postnatal blood lead concentrations and school-aged IQ should also apply to protection of the fetus and, by extension, to women of childbearing age.

3.2 EVIDENCE OF DEVELOPMENTAL NEUROTOXICITY FROM EPIDEMIOLOGICAL STUDIES

As discussed above, the developmental neurotoxicity of lead has been established via evidence from a range of health endpoints, including demonstrated adverse effects on behaviour, academic achievement, reaction time, motor skills, balance, and sensory acuity. However, the potential effect of lead exposure on psychometric tests of intelligence (IQ) among school-aged children was selected as the critical endpoint for derivation of a TRV for the developmental neurotoxicity of lead. IQ was

selected as the critical developmental endpoint because, of all the developmental neurotoxicity endpoints that have been examined, IQ has the greatest strength of evidence of an adverse effect. IQ has also been reliably related to latter life outcomes, such as academic achievement and earning potential and lead's potential adverse effects on children's IQ can, therefore, be expressed via potential impacts on these more tangible outcomes. It is important to keep this point in mind when contemplating the implications of the potential effects of lead exposure on IQ of children: IQ is a surrogate for many other neurological outcomes and adverse consequences beyond the immediate implications of reduced performance on psychometric tests of intelligence.

As a global endpoint IQ has specific advantages and disadvantages. Because the precise systems and functions that are potentially affected by a particular timing and dose of lead exposure remain uncertain, examination of responses in a global measure of neurological functioning, such as IQ, allow for a broad survey of potential effects. On the other hand, this lack of specificity means that IQ tests may be insensitive to significant adverse effects on specific components or domains of neurodevelopment and functioning. It is important to keep this point in mind when reviewing the evidence of the potential effects of lead exposure on IQ of children: IQ is a global, but potentially insensitive measure of the potential developmental neurotoxicity of lead.

IQ decrements among school-age children (up to 18 years old) were selected as the critical endpoint for derivation of a TRV for the developmental neurotoxicity of lead. While there is evidence of adverse effects of lead exposure on early life psychometric tests of neurological development, such as the Bayley Scales of Infant Development (BSID), this endpoint was rejected as a critical endpoint for the following reasons: (1) the relative strength of the evidence supporting adverse effects on this endpoint is not as strong as that of IQ in school-aged children; and (2)

early life psychometric tests of neurological development are less well correlated with latter life outcomes than school-aged IQ and therefore the adverse consequences of reported effects on early life psychometric tests of neurological development are less certain. Therefore, the results of lead exposure effects on early life psychometric tests of neurological development that are presented below are provided for completeness only and in support of the evidence of adverse effects on IQ of school-aged children.

Early life lead exposure, as measured by blood lead concentrations, has been associated with deficits in academic achievement and psychometric tests of intelligence out to at least 17 years old. For example, Ris et al. (2004) demonstrated that blood lead concentrations measured in subjects of the Cincinnati Lead Study (CLS) at six and a half years old were related to deficits in IQ, academic achievement and other dimensions of neurological functioning (attention, visioconstruction, and fine-motor skills) at 15 to 17 years of age. Significant inverse associations between early life blood lead and covariate adjusted IQ were also reported to at least 10 years of age among subjects of the Mexico City Prospective Lead Study (Schnaas et al., 2000), Yugoslavia Prospective Lead Study (Wasserman et al., 2003), Port Pirie, Australia cohort (Tong et al., 1996), and Boston, USA cohorts (Bellinger et al., 1992). Therefore, the endpoint is explicitly defined as IQ deficits in school-aged children (i.e. up to age 18). However, it should be noted that there is currently no evidence that decrements in school-aged IQ do not persist throughout life. Additionally, academic, behavioural and social challenges which manifest in school-aged children have potentially life-long health and socioeconomic implications.

The epidemiological association between early life lead exposure and IQ test scores is perhaps the most well studied of all potential environmental health effects. The time and effort in presenting a review of every epidemiological publication on this association would be significant. This effort had to be balanced against the diminishing returns of reviewing evidence that may be considered redundant. The approach used in this report is to present what is considered to be a representative sample of the larger literature and draw conclusions based on this sample. This truncated approach is consistent with recent reviews and risk assessments of other health agencies, such as the US EPA (2006), US ATSDR (2007), IARC (2006) and WHO (1995). Therefore, only a complete review and analysis of the longitudinal studies, pooled analysis, and meta-analysis of the relationship between blood lead and children's IQ is presented herein. Studies emphasized include those that are recent, multi-cohort (meta-analysis or pooled analysis), reporting exposure ranges that are representative of contemporary environmental exposures, or those that examined study cohorts that are closely representative of the Canadian population. It is not thought that the conclusions of this review would change significantly by including the many cross-sectional studies of this endpoint. A number of crosssectional studies are reviewed in the sections below on the extent and shape of the dose-response relationship as well as variability in biomarker-response because some cross-sectional studies provide important evidence on these issues.

Cross-sectional studies are not afforded the same weighting as longitudinal studies because temporality cannot be established in cross-sectional studies. Also, a single measure of blood lead levels is more likely to be subject to exposure misclassification. In this respect, cross-sectional studies have less power to detect a dose-response relationship. For these reasons evidence from cross-sectional studies is not reviewed in detail here. Readers are referred to (WHO, 1995; US ATSDR, 2005; US EPA, 2006) for comprehensive reviews of cross-sectional studies.

3.2.1 Longitudinal Studies of Early Life Lead Exposure and School-Aged IQ

Longitudinal studies of early life exposure and potential effects on psychometric test scores may be considered superior to cross-sectional studies in that both exposure and outcome are followed over time. This reduces the potential for exposure misclassification and also addresses the issue of temporality (i.e. exposure precedes effect). Another advantage, as a whole, that the longitudinal studies of early life lead exposure and the potential effects on psychometric test scores have over the collection of cross-sectional studies is that many of the longitudinal studies used comparable methods of assessment and analysis. This facilitates more legitimate and informative comparisons of results across studies as well as makes an analysis of the pooled data feasible and meaningful.

Longitudinal studies of early life lead exposure and potential effects on psychometric test scores include several biomarkers of lead exposure and outcomes. Biomarkers of exposure that have been investigated include maternal blood lead during pregnancy or at delivery, maternal bone lead, blood collected from the umbilical cord at delivery (cord lead), and postnatal children's blood lead – which may be collected via venous or capillary sample. Outcomes that have been assessed include early life psychometric tests of neurological development, such as the Bayley Scales of Infant Development (BSID) and psychometric tests of intelligence (IQ), such as the Wechsler Intelligence Scales for Children (WISC) or equivalent. For completeness the review that follows includes a discussion of all these biomarker-endpoint combination for which there is the greatest strength of evidence of adverse effects and the one that is was selected for development of a TRV for the protection against adverse developmental neurological outcomes is blood lead in children and school-aged IQ.

As of January 1st 2008 there were 12 published longitudinal studies of early life lead exposure and potential effects on psychometric test scores and also a pooled

analysis of the results of seven of the eight of these studies that had been initiated prior to 1995. A review of the methods and results of each of these studies is provided below. In summary, this body of evidence is strongly suggestive, but not consistently supportive, of an adverse association between early life chronic lead exposure (as measured by various biomarkers) and decrements in school-aged children's IQ, after correcting for covariates. Table 13 presents a summary of the results of these studies and very general information of the relative cohort demographics and lead exposure levels among the studies. This association has been studied in a wide variety of ethnic and socioeconomic populations across a wide range of lead exposures. Four of the 12 longitudinal studies and the international pooled analysis are strongly supportive of an association between early life lead exposure and potential effects on psychometric test scores. Six of the 12 longitudinal studies report inconsistent results across biomarker of lead exposure, time point of exposure or assessment, or neurological assessment tool. Two of the 12 longitudinal studies are strongly negative.

Results	Relative lead exposure	Relative socio- economic stratum	n ¹	Cohort	References
+	low	low	174	Rochester, NY USA*	Canfield <i>et al</i> ., 2003; Jusko <i>et al</i> ., 2008
±	high	NR	133	Shanghai, China	Shen <i>et al</i> ., 1998
+	variable	high	392	Kosovo*	Waserman <i>et al</i> ., 1992, 1994, 2003; Factor- Litvak <i>et al</i> ., 1999
+	high	low	780	Treatment of Lead Exposed Children (TLC) Trial, USA	Chen <i>et al.</i> , 2005
±	high	low	297	Cincinnati, USA*	Dietrich <i>et al.</i> , 1987, 1991, 1992, 1993; Ris <i>et</i> <i>al</i> ., 2004
±	low	high	201	Boston, USA*	Bellinger <i>et al.</i> , 1984, 1986, 1987, 1991, 1992; Bellinger and Needleman, 2003
-	high	low	212	Cleveland, USA*	Ernhart <i>et al</i> ., 1987, 1989; Greene <i>et al.</i> , 1992, 1993
+	high	high	595	Port Pirie, AUS*	Wigg <i>et al.</i> , 1988; McMichael <i>et al.</i> , 1988; Baghurst <i>et al.</i> , 1992; Tong <i>et al.</i> , 1996, 1999.
-	low	high	274	Sidney, AUS	Cooney <i>et al</i> ., 1987, 1989, 1991.
±	low	high	150	Mexico City Prospective Study*	Schnaas <i>et al</i> ., 2000, 2006
±	low	low	197	Lead and Fetal Neurodevelopmental Study, Mexico City	Gomaa <i>et al</i> ., 2002
±	low	low	294	2 Cohort Study, Mexico City	Téllez-Rojo <i>et al.</i> , 2006
+	variable	variable	1,303	International Pooled Analysis	Lanphear <i>et al</i> . 2005

 Table 13. Summary of 12 longitudinal studies and pooled analysis of early life lead exposure and potential effects on psychometric test scores.

+ strong evidence of a significant inverse relationship between early life lead exposure and IQ

± equivocal evidence of a significant inverse relationship between early life lead exposure and IQ

- no evidence of a significant inverse relationship between early life lead exposure and IQ

1: highest n among multiple analyses

*included in the International Pooled Analysis

The studies reviewed below are presented in alphabetical order according to city or geographical location of the study cohort.
Detailed summary tables of each of the longitudinal studies of early life lead exposure and psychometric tests of neurological development and intelligence are presented in Appendix B. For each longitudinal study cohort, these tables list the references for each published report, the age and endpoint for each assessment of outcome, the various lead exposure indices and associated summary statistics, covariates investigated, study exclusion criteria, and the quantitative results of each published report.

Boston, MA USA

The Boston cohort consisted of 249, mostly Caucasian (95%), mixed sex, socially and economically advantaged children with relatively low lead exposures born at an urban hospital in Boston in 1979 or 1980. Both maternal and subject IQs were well above average. Lead exposure was measured as cord blood and blood lead at 6, 12, 18, 24, 57, and 120 months (10 yrs). Neurodevelopmental effects were assessed concurrently. A broad variety of potential social, economic and environmental covariates were examined for the Boston cohort, including family social class, maternal IQ, Caldwell-Bradley Home Observation for Measurement of the Environment (HOME) score at 6 and 24 months, and serum ferritin. Children with a medical condition known to increase risk of developmental problems (Down's syndrome, cleft palate, gestational age < 34 weeks, and retinoblastoma) and non-English speaking families were excluded from the study. Postnatal blood lead up to two years of age was measured via capillary samples.

Neurological development among 249 children from the Boston cohort was assessed with the Bayley Scales of Infant Development (BSID) semi-annually between the ages of 6 months and 2 years (Bellinger *et al.*, 1984; Bellinger *et al.*, 1986; Bellinger *et al.*, 1987). BSID scores were not associated with any of the

postnatal blood lead measures, but poorer performance, after adjusting for covariates, was observed for those infants with cord blood lead concentrations > 10 μ g/dL, relative to those with cord blood lead of 6-7 μ g/dL or < 3 μ g/dL. Additional analysis of these data revealed that cord lead and postnatal blood lead had a greater negative effect on BSMD test scores for children from lower SES strata (Bellinger *et al.*, 1988).

Intellectual function in 170 subjects of the Boston cohort was assessed with the McCarthy Scales of Children's Abilities at about 5 years of age (Bellinger *et al.*, 1991). Mean blood lead levels at 5 years were 6.4 (SD 4.1) μ g/dL. General Cognitive Index (GCI) scores of the McCarthy Scales of Children's Abilities were, after adjusting for covariates, significantly inversely associated with blood lead at age 2, but not blood lead at other ages nor mean blood lead integrated over a range of ages. The concentration of lead in the dentine of shed deciduous teeth was not significantly associated with intellectual performance after adjustment for confounding.

The most recent assessment of the Boston cohort was done when the subjects reached 10 years of age (Bellinger *et al.*, 1992). One hundred and forty eight subjects were assessed with the Wechsler Intelligence Scales for Children-Revised (WISC-R) and the Battery Composite scores on the Kaufman Test of Educational Achievement-Brief Form (K-TEA). Mean blood lead levels at 10 years were 2.9 (SD 2.4) μ g/dL. A significant negative association was reported between blood lead at 24 months and adjusted WISC-R full scale and verbal IQ. The negative association between these endpoints and blood lead at other time periods (cord and 6, 12, 18 and 57 months) was not significant after adjusting for covariates. The dose-response relationship was modeled as a linear relationship with an estimated decrement of 0.58 (0.17-0.99) in full scale IQ points at 10 years of age associated with a 1 μ g/dL increase in blood lead at age two. The authors note that the reduced sample size

and the reduced range of blood lead values decreased the power of the study to detect effects at 10 years of age.

A reanalysis of the Boston cohort data using nonparametric smoothing revealed that the inverse association between IQ at 10 years and two year blood lead concentrations persisted at blood lead levels below 5 µg/dL, with no evidence of a threshold (Schwartz, 1994a).

Bellinger and Needleman (2003a) also reanalyzed data on 48 children from this cohort whose measured blood lead concentrations never exceeded 10 μ g/dL. The association between blood lead at age two and IQ decrements at age 10 remained statistically significant and the slope of the dose-response relationship was approximately three-fold steeper for those whose peak blood lead never exceeded 10 μ g/dL. Note that this reanalysis was published as correspondence and was not subject to publisher peer review.

Cincinnati, OH USA

The Cincinnati Lead Study (CLS) is a birth cohort of approximately 300, lower socioeconomic status, predominantly African-American subjects that were followed from birth until 15 to 17 years of age. Lead exposure was relatively high in this cohort; blood lead of 95% of subjects exceeded 10 μ g/dL sometime during the first five years of life. Blood lead measurements were made on a quarterly basis from 10 days postnatal until 5 years of age and again at 5.5, 6 and 6.5 years of age. Maternal blood lead was also measured at the time of the first clinical visit after recruitment (50% 1st trimester, 49% 2nd trimester, 1% last trimester).

Medical and neurological assessments were made on a quarterly basis until five years of age and again at 5.5, 6, 6.5, 10, and 15-17 years of age. Socioeconomic

data were collected on a regular basis. Thirty five covariates were examined as potential confounders. These included maternal and child medical conditions, maternal IQ, family SES, quality of the caregiver environment (HOME score measured at 6,12, 24, and 36-48 months), maternal alcohol, drug and tobacco use; subject birth measurements, Fe status, medical conditions, and adolescent alcohol, tobacco, and marijuana use. Exclusion criteria included mother's addicted to drugs or alcohol, with diabetes or neurological disorders, psychoses, or mental retardation and subjects < 35 weeks gestation, < 1,500 g birth weight, or genetic syndromes or other serious medical conditions at birth.

Dietrich *et al.* (1987) reported on associations between maternal, cord blood and infant blood lead and Bayley Scales of Infant Development Mental Development Index (MDI) scores at three and six months. Prenatal (maternal blood lead) and umbilical cord blood lead values were inversely associated with covariate-adjusted MDI at three months. No significant associations were reported for 10 day or concurrent blood lead and three month MDI. Prenatal (maternal blood lead) and 10 day infant blood lead values were inversely associated with covariate-adjusted MDI at 6 months, with a stronger effect observed for lower SES and male subjects. No significant associations were reported for cord lead or three month blood lead and 6 month MDI. Structural equation models were used to test the hypothesis that leadinduced neurodevelopmental effects at three and six months of age were due, in part, to lead-induced lower birth weight and reduced gestation age. Maternal blood lead was used as the measure of lead exposure. These analyses indicated that *in utero* lead exposure may have exerted additional effects on neurodevelopment due to its negative effects on fetal growth and maturation.

Neurological outcomes in 297 children from the Cincinnati cohort were assessed with the Bayley MDI again at age two (Dietrich *et al.*, 1990). No statistically significant inverse relationships between 11 indexes of prenatal or postnatal blood

lead and 24 month Bayley MDI scores were reported. Prenatal blood lead was found to have a significant positive effect.

Dietrich *et al.* (1991) reported on the results of neurocognitive assessment of 247 children from the Cincinnati cohort at age four. Intellectual functioning was assessed with the Kaufman Assessment Battery for Children (K-ABC). The K-ABC is nationally standardized and is comparable to other measures of early childhood intellectual functioning such as the McCarthy Scales of Children's Abilities. The K-ABC Mental Processing Composite (MPC) standard score is interpretable as an intelligence quotient (IQ) score. Neonatal (~ 10 days postnatal) blood lead was significantly and inversely related to all adjusted K-ABC subscale scores. However, the significance of this association was retained only for those subjects below the median socioeconomic status when interaction between neonatal blood lead and SES was introduced into the model. After adjusting for covariates, no other measures of prenatal or postnatal blood lead retained a significant association with K-ABC scores. The results from Dietrich et al. (1991) that suggest lower SES children may be more vulnerable to the neurodevelopmental effects of lead exposure are supported by *in vivo* evidence that animals in an enriched environment may be less susceptible or better able to recover from the adverse neurodevelopmental effects of early life exposure to neurotoxins (Schneider et al., 2001; Hannigan et al., 2007).

Neurological outcomes in 259 children from the Cincinnati cohort were again assessed with the K-ABC at age five (Dietrich *et al.*, 1992). In contrast to the results from the four year old performance on the K-ABC, no significant associations were reported between K-ABC scores and neonatal blood lead (the authors did not explore a sex or SES interaction in this paper). Similar to the results from the four year assessment, with the exception of one K-ABC subtest score and one measure of lead exposure (Simultaneous Processing standard score and mean blood lead at age four), no significant association was found between measures of postnatal blood lead and K-ABC scores.

Neurodevelopmental effects were assessed using WISC-R amongst 253 subjects of the Cincinnati at 6.5 years of age (Dietrich *et al.*, 1993b). Significant negative associations between postnatal blood lead (measured at 5 and 6 years) and adjusted full scale IQ were reported. No significant association was reported for lifetime average or maternal blood lead, neonatal blood lead nor average blood lead for years one through four.

Ris *et al.* (2004) re-examined the Cincinnati cohort at 15-17 years of age. A comprehensive neurodevelopmental test battery was administered and patterns of results were analyzed using a principal components factor analysis. Seventy eight month (6.5 years) blood lead was significantly and inversely associated with lower Learning/IQ factor scores after adjusting for covariates, whereas no significant association was reported between this endpoint and prenatal blood lead or average childhood (up to 5 years) blood lead. The Learning/IQ factor was comprised of scores from the reading, spelling and arithmetic subtests from the Wide Range Achievement Test-3rd Edition (WRAT-3) and the Block Design Subtest and the Vocabulary Subtest from the WISC-III. Significant inverse associations were also reported between Attention factor scores and prenatal and average childhood lead (males only); between Visioconstruction factor scores and 78 month blood lead.

Assessments at both earlier and latter periods of development of the Cincinnati cohort suggest that lower SES subjects are more sensitive to the neurodevelopmental effects of lead (Dietrich *et al.*, 1987; Dietrich *et al.*, 1991; Ris *et al.*, 2004).

Cleveland, OH USA

The Cleveland, OH cohort consisted of 359 subjects, approximately 35% African-American, mixed sex, lower SES mother-infant pairs recruited into a study to examine the effects of fetal alcohol exposure. Of the mothers recruited, 50% had histories of problems associated with alcohol use (as determined by the Michigan Alcoholism Screening Test). Maternal blood lead was sampled at delivery or one day post partum. Cord blood was also sampled. Venous blood samples were obtained from the children at 6 months and 2, 3 and 4.8 years of age. Circumpulpal dentin lead concentrations were also quantified from shed deciduous incisors collected from 164 subjects between 5 and 7 years old. Covariates investigated included maternal IQ, alcohol, smoking and drug use, parental education, Authoritarian Family Ideology (AFI), subject sex, age at testing, birth order, ethnicity, and home and preschool HOME Inventory scores (at 1,2, 3 and 4.8 years old). Exclusion criteria included < 37 weeks gestation, neonatal intensive care, maternal use of narcotics, English as a second language, and maternal schizophrenia.

Ernhart *et al.* (1987) reported the associations between maternal, cord and postnatal blood lead with psychometric test results for 285 subjects up to 3 years of age. Neurological development was assessed with Bayley MDI scores at 6 months, and 1 and 2 years of age. A modified version of the Kent Infant Development Scale (KID) was also administered at 6 months. The KID Scale is based on parental reporting of infant achievement in 5 domains. The S-BIS was administered at 3 years of age. Linear regression models were used to assess the association between blood lead measurements and psychometric test scores. The authors reported the unadjusted correlation coefficients between the blood lead metric and outcome of interest, as well as the variance attributed to covariates retained in the model, the variance attributable to lead alone, and the significance of the variance attributed to lead. Maternal blood lead was reported to make a significant contribution to the variance

of the observed inverse relationship between maternal blood lead and six month MDI, PDI and KID scores. Significant adverse associations of blood lead and psychometric testing were not reported for any other blood lead measurement or psychometric test outcome.

Ernhart *et al.* (1989) reported on the assessment of 242 children in the Cleveland cohort who were assessed with the WPPSI at 4.8 years old. Preschool period medical history (e.g. *otis media*, failure to thrive) and psychological stress (e.g. hostile parental separation, abuse) were also assessed and evaluated as potential confounders at this time point. Preschool medical problems were significantly and negatively associated with IQ. There were no other significant relationships between these covariates and measures of blood lead or IQ. Correlations of maternal and child IQ were compared between high and low maternal, cord and mean preschool blood lead groups. The correlation coefficients between maternal IQ and child IQ were lower in the high maternal blood (> 8 μ g/dL) and cord blood lead (> 8 μ g/dL), but these differences did not reach statistical significance. Using multiple linear regression, no significant associations were reported between maternal, cord or preschool mean blood lead and IQ at 4.8 years old after adjusting for covariates.

Greene *et al.* (1992) used data from the Cleveland cohort to analyze the contributions of various risk factors to elevated blood lead and psychometric tests scores. This analysis demonstrated the strong relationship between measures of the quality of the care-giving environment (HOME Inventory or other measures) and both psychometric test scores and blood lead concentrations in preschool-aged children. Of the many socioeconomic and demographic variables included in this analysis, the HOME Inventory score was the best predictor of preschool IQ and was also very strongly correlated with blood lead.

The associations between dentine lead and IQ scores at 4.8 years were also examined in this cohort (Greene and Ernhart, 1993). Shed deciduous incisors were collected from 164 subjects between 5 and 7 years old. Lead was quantified in the circumpulpal region of the dentin. Dentin lead was square root transformed – the square root transformation was used as an intermediate approach, less severe than log transformation, to normalize the distribution of the dentin lead values. Using multiple linear regression, dentine lead was inversely associated with IQ at 4.8 years old, but the relationship was not statistically significant (p=0.063).

Greene and Ernhart (1993) also used an errors-in-variables regression framework to explore the implications of potential non-constant measurement errors in dentine lead as well as potential measurement error of key sociodemographic covariates (HOME and maternal IQ). Measurement error in dentine lead was modeled as a function of dentine lead and the number of teeth sampled per subject. The results of this modeling demonstrated that ignoring measurement error in the lead exposure variable will be null biasing (underestimate the magnitude of lead effect) and will over-estimate the precision of the lead effect. The effect of ignoring potential measurement error in dentin lead is illustrated in Figure 3. This analysis also demonstrated that ignoring potential measurement error in covariates will overestimate the magnitude of the lead effect. The sensitivity of linear regression analysis to assumptions of no measurement error in exposure and covariates was illustrated. If no measurement error is assumed, the modeled loss in IQ associated with a change in dentine lead from the 10th to the 90th percentile was -4.5 (95% CI -9.2 to + 0.2) IQ points. When modeled with plausible levels of measurement error assumed for dentine lead, HOME score and maternal IQ, the modeled loss in IQ associated with a change in dentine lead from the 10th to the 90th percentile was -5.2 (95% CI -12.0 to + 1.6) IQ points.



Figure 3. Reproduced from Greene et al., (1992). Sensitivity of the magnitude of the linear regression coefficient and associated 95% confidence interval for the relationship between dentine lead (PbD) and IQ among subjects of the Cleveland longitudinal lead study cohort at 4.8 years old. The parameter $^{\delta}$ PbD determines the size of the assumed measurement error in PbD by the following: variation in "true" PbD = ($^{\delta}$ PbD /n_{teeth})(measurement error in "true" PbD). The estimate of $^{\delta}$ PbD = 0.38 was obtained using the data of 20 children who supplied multiple tooth samples. The model included maternal IQ, maternal education, date of 1st antenatal visit, and subject ethnicity, preschool medical history, age at testing and preschool HOME score.

Mexico City Prospective Lead Study, Mexico City, Mexico

A sample of 321 mother-infant pairs born at the National Institute of Perinatology in Mexico City were recruited for study at the 2nd trimester of pregnancy and children were followed for up to 10 years postpartum. The sample consisted of lower SES (mean maternal IQ 93), moderately exposed (geometric mean maternal blood lead

during pregnancy was 8.0 µg/dL) subjects. Maternal blood lead was measured every 8 weeks of pregnancy, starting at week 12. Child blood lead (venous) was measured every 6 months until age 5 and annually thereafter until 10 years of age. Neurological development was assessed at 48 hours, 15 and 30 days using the Brazelton Neonatal Behavioural Assessment Scale (NBAS); from 3-5 years with the McCarthy Scales of Children's Abilities (MSCA) General Cognitive Index (GCI), and from 6-10 years using the WISC-R. Covariates examined included maternal IQ, family SES, quality of the caretaker environment (HOME scale), subject 5 min Apgar score⁵, sex, birth weight, and birth order. Covariates not examined include maternal drug, alcohol and tobacco use and subject medical history. HOME scale was measured only before 6 months postpartum and may have limited relevance to latter measures of IQ. Exclusion criteria included: maternal age < 15 or > 45 years, habitual use of drugs or alcohol, use of prescription medications, psychosis, or medical conditions including hypertension, diabetes, hepatitis, or toxoplasmosis and subject <36 weeks gestational age, 5 min Apgar < 6, birth weight < 2000g, multiple births, and the presence of medical conditions or congenital anomalies.

Rothenberg *et al.* (1989) reported on a pilot study of the relationships between prenatal and perinatal blood lead and neurological development of 42 neonatal subjects. Development was assessed with the NBAS administered at 48 hours and 15 and 30 days postnatal. Various significant associations were reported, but they were not consistent in direction of effect.

Schnaas *et al.* (2000) reported on the results of 112 children who were assessed at 6 month intervals between 3 and 5 years of age using a Spanish translation of the McCarthy Scales of Children's Abilities. The authors reported a high degree of

⁵ The Apgar score is a simple method of rapidly assessing the health of a newborn. It is determined by evaluating the neonate on five criteria (Appearance, Pulse, Grimace, Activity, **R**espiration) on a scale from zero to two, then summing up the five values thus obtained. The resulting Apgar score ranges from zero to 10.

collinearity between HOME scores and maternal IQ and, therefore, scores were only corrected for maternal IQ. A series of cross-sectional linear regression models were constructed to analyze the relationships between various indices of blood lead and GCI scores at various time points. The authors reported no significant relationship between GCI scores and any of the prenatal or perinatal blood lead measures. However, the 24-36 month mean blood lead was significantly negatively associated with adjusted GCI scores at 48 months and the 42-54 month mean blood lead was significantly negatively associated with adjusted GCI scores at 54 months. The results for the postnatal blood lead results are presented in Figure 4 below. A general linear model for repeated measures was also used to evaluate lead related changes in individual GCI scores with age. Planned polynomial contrasts were used to test within-subject effects (variation of lead effect with GCI at various ages). Significant within subject effects (lead effect on GCI with age) were reported for 6-18 month blood lead and 24-36 month blood lead. The effect of 6-18 month blood lead was increasingly negative with age. The effect of 24-36 month blood lead was increasingly negative with age until 48 months and then returned to baseline.





Figure 4. Reproduced from Schnass et al. (2000). Summary of the effects of postnatal blood lead on General Cognitive Index assessed semi-annually from 36 to 60 months of age among 112 subjects of the Mexico City Prospective lead Study. The solid line represents effects on GCI of mean 6-18 month blood lead. The long dashed line (----) represents effects on GCI of mean 24-36 month blood lead. The short dotted line (....) represents effects on GCI of mean 42-54 month blood lead. Whiskers represent standard errors of the adjusted regression coefficients. Points are off-set on the time axis for clarity only. + = p < 0.10; ++ = p < 0.05

Analysis of most cohort studies did not include a formal repeated measures design and instead employed repeated, but separate cross-sectional analysis of the outcome at various ages. Exceptions include (Bellinger *et al.*, (1987; 2000); Wasserman *et al.*, (2000a); and, Tellez-Rojo *et al.*, (2006)).

Full scale IQ was measured annually in 175 subjects from age 5 to 10 years using a Spanish version of the Wechsler Intelligence Scale for Children-Revised (WISC-R) (Schnaas *et al.*, 2006). Using linear mixed models with random intercept and slope to analyze the pattern of lead effect on IQ, third trimester (week 28 only) maternal blood lead levels were found to be significantly associated with IQ at age six through 10 years after controlling for other measures of blood lead and other covariates. The authors report that there was no evidence that the 3rd trimester blood lead effect on 6-10 year-old IQ was mediated by lead effect on birth weight. The 6-10 year average blood lead initially indicated a significant inverse relationship with 6-10 year IQ, but this association lost significance when other measures of blood lead were introduced into the model; however, the reduction in the coefficient was not accompanied by an increase in the variance associated with 6-10 year average blood lead and this variable might have retained its significance with the increased power of a larger sample size. Cross-sectional associations between IQ and blood lead measured at or averaged over different time points were not significant (Schnaas *et al.*, 2006).

This cohort is unique because of the number of maternal blood lead measurements taken during pregnancy (four). Only one other study -the Port Pirie, Australia cohort-took multiple maternal blood lead samples during pregnancy; however, that study did not analyze the data from different time points during pregnancy (Baghurst *et al.*, 1992). All other studies of maternal blood lead during pregnancy and child neurodevelopment outcome either relied on a single maternal blood lead measure or cord blood lead at delivery.

The analysis by Schnaas *et al.* (2006) also deserves special attention for simultaneously including all measures of blood lead in their analysis. Because the unique exposure pattern in this cohort was idiosyncratic and intermittent (lead from ceramic flatware), collinearity among the lead measures was not significant. Collinearity among highly correlated lead variables in the same linear model will produce biased estimates of the lead effect and inflated standard errors (Schnaas *et al.*, 2006). Schnaas *et al.* (2006) reported that maternal blood lead between 2nd and 3rd trimester was only moderately correlated (Pearson correlation of 0.48) and that maternal blood lead during pregnancy and postnatal child blood lead was poorly correlated (<0.23).

Schnaas *et al.* (2006) used the *J*-test to determine whether their observed concentration-response relationship between 28 week maternal blood lead and child IQ at 6-10 years is best described as a linear relationship or a \log_{e} -linear relationship. The logarithmic form of the relationship provided a better fit to the data (*p*=0.02).

One of the reports from this cohort study was not reviewed because it was published in Spanish (Schnaas *et al.*, 1999).

Lead and Fetal Neurodevelopment Study, Mexico City, Mexico

Gomaa *et al.* (2002) examined the effects of prenatal and postnatal lead exposure on the neurodevelopment of 197 low to middle-class, mixed sex children from Mexico City. Gomaa *et al.* (2002) is the only published study of the neurodevelopmental effects of lead located that includes maternal bone lead as a measure of perinatal lead exposure. Lead was measured in cord blood. Maternal bone lead (tibia and patella) was measured *in* vivo using ¹⁰⁹Cd K shell X-ray fluorescence (K-XRF) within 4 weeks postpartum. Postnatal blood lead (venous) was sampled at 12 months and 24 months of age. Neurological function was assessed at 24 months using a Spanish version of the Bayley Scales of Infant Development II (BSID-II). Both the Mental Development Index (MDI) and the Psychomotor Development Index (PDI) were scored. Study exclusion criteria included an extensive list of relevant medical conditions, intention not to breastfeed, gestational age < 37 weeks, 5 minute Apgar < 6, or birth weight < 2000 g. Covariates examined included maternal age, IQ, education, and marital status; paternal education; and infant sex, gestational age, medical history and duration of breastfeeding. Covariates did not include an assessment of maternal smoking, alcohol or drug use, socioeconomic status, the quality of the care-giving environment, nor nutritional status. Because of the importance of these potential confounders the results of Gomaa *et al.* (2002) are given reduced weighting in this report.

Postnatal blood lead was not significantly associated with adjusted MDI or PDI scores. Maternal patella lead and cord blood were significantly and negatively associated with adjusted MDI scores. Maternal tibia lead was negatively associated with MDI scores, but the relationship was not significant after adjusting for confounders. Maternal patella lead was only significantly and negatively associated with adjusted MDI scores when treated as a categorical variable (quartiles); it was not significantly (p=0.07) associated with MDI as a continuous variable.

Two Cohort Study, Mexico City, Mexico

Tellez-Rojo *et al.* (2006) examined the relationship between lead exposure and neurological functioning in toddlers from Mexico City whose measured blood lead concentration never exceeded 10 μ g/dL. The 294 subjects were drawn from two cohorts: (1) the Mexico City Lead and Fetal Neurodevelopment Study: and (2) a second, unnamed cohort of subjects born between May 1997 and July 1999 at the

same hospitals where subjects were recruited for the Mexico City Lead and Fetal Neurodevelopment Study. Blood lead measurements were made at 12 and 24 months. Neurological functioning was assessed at both time periods using a Spanish version of the Bayley Scales of Infant Development II (BSID-II). Both the Mental Development Index (MDI) and the Psychomotor Development Index (PDI) were scored. Exclusion criteria included maternal alcohol consumption, addiction to illicit drugs, and relevant medical conditions. Covariates examined included maternal IQ and parental education, but did not include a direct measure of socioeconomic status, the quality of the care-giving environment, nor nutritional status. Because of the importance of these potential confounders the results of Tellez-Rojo *et al.* (2006) are given reduced weighting in this report.

Mixed-effects regression models with random intercept were used to examine the longitudinal association between blood lead and BSID-II test scores at 12 and 24 months. No significant association was reported for blood lead and neurodevelopmental outcomes at 12 months of age. However, a significant negative association was reported when the subjects reached 24 months of age. The strength of this association was largely unchanged when 12 month blood lead was also considered in the model. The association at 24 months was largely attributed to the subjects in the 2nd cohort, whose blood lead at 24 months was approximately 30% lower than subjects from the Mexico City Lead and Fetal Neurodevelopment Study cohort. A steeper dose-response curve at lower lead exposures was also reported for the results of the pooled cohort.

Port Pirie, Australia

Seven hundred and twenty three mother-infant pairs were recruited from 1979-1982 from Port Pirie, Australia – the site of one of the largest lead smelters in the Southern hemisphere. Children from this cohort had relatively high blood lead

concentrations (the geometric mean of lifetime average blood lead to 11-13 yrs was 14.1 µg/dL) and were of middle class. Blood lead was sampled during pregnancy (14-20 weeks gestation and early 3rd trimester) and at delivery (cord). A capillary blood sample was collected from the children and at age 6, 15, and 24 months and annually thereafter to seven years. A venous blood sample was collected from the children at 11-13 years. Psychometric testing was administered when subjects were two (BSID), four (MSCA), seven and 11-13 years (WISC-R) old. Covariates examined included parental occupational prestige, smoking habits, and education; maternal psychological status, IQ, length of residence in Port Pirie, age at delivery, infant feeding method, and marital status; and subject's family functioning, quality of the care-giving environment (HOME score) at three years, iron status, sex, age at testing, school grade, family size, life events, birth order, birth weight, pharmaceutical use, and prolonged school absences. Subject tobacco, drug or alcohol use does not appear to have been examined. Lead exposure among this cohort of children was relatively high - the geometric mean blood lead increased from 8.3 µg/dL at birth to 21.2 µg/dL at two years and decreased to 7.9 µg/dL by the age of 11-13 years.

Wigg *et al.* (1988) reported on a multiple linear regression analysis of the association between prenatal, cord and postnatal blood lead among 595 subjects from the Port Pirie cohort and BSID MDI scores at two years of age. After adjusting for covariates, there were no significant associations between any of the measures of lead exposure and BSID MDI scores at two years of age.

McMichael *et al.* (1988) reported on the association between the general cognitive index (GCI) of the McCarthy Scales of Children's Abilities measured in 537 four year old subjects of the Port Pirie study cohort and various indices of prenatal and postnatal blood lead. Postnatal, but not prenatal nor cord blood lead, was significantly inversely associated with adjusted GCI at age four. The strongest relationship, among many indices of lead exposure, and adjusted GCI was between integrated postnatal average (area under the curve) and GCI. Blood lead at six months, 24 months, and 36 months was also significantly inversely associated with adjusted GCI at 48 months, but not blood lead at 15 months nor concurrent blood lead. There was no significant relationship reported between the change in a subject's blood lead from the first two years and the second two years of life to change in (rank-ordered) measures of cognitive ability (BSID at 2 years and MSID at 4 years).

Baghurst *et al.* (1992) reported on the association between IQ (WISC-R) measured in 494 seven year old subjects from the Port Pirie cohort and various indices of prenatal and postnatal blood lead. After adjusting for covariates, significant inverse associations were reported for postnatal, but not prenatal or cord blood lead and IQ at age seven. In a stratified analysis, girls were more sensitive to the effects of lead than were the boys. The slope of the log-linear regression between lifetime average blood lead and adjusted IQ at seven years was about three-fold steeper for the girls.

Tong *et al.* (1996) reports on the association between IQ (WISC-R) at 11 to 13 years of age and various measures of blood lead for 375 children from the Port Pirie cohort. Postnatal blood lead from three years on, both concurrent and lifetime average were significantly negatively associated with adjusted IQ at 11-13 years of age. An exception was lifetime average blood lead to age 7 – where *p*=0.06. The greatest effect size was reported for the association with integrated lifetime average blood lead to the age of 11-13 years. The linear estimate, across the range of lifetime average blood lead to the age of 11-13 years, of this effect size would be equivalent to a -0.3 (95% CI: -0.6, -0.01) IQ point decrement per 1 μ g/dL increase in integrated lifetime average blood lead. Stratified analysis revealed that the association between lifetime average blood lead and IQ was stronger for females than males – the adjusted regression coefficient was about 3 fold higher for females.

No significant association was reported between IQ and maternal nor cord blood lead.

Tong *et al.* (1998) further evaluated data from the Port Pirie cohort to examine the relationship between intra-individual changes in blood lead and intra-individual changes in measures of cognitive development. No statistically significant longitudinal association between declining blood lead concentrations and increasing psychometric test scores was observed.

Rochester, NY USA

Canfield *et al.* (2003a) report on a longitudinal prospective study of 172 mostly (73%) African-American mixed sex socially and economically disadvantaged children from Rochester, NY. Lead exposure was estimated from blood lead measurements at 6, 12, 18, 24, 36, 48, and 60 months of age. Cognitive functioning was measured with the Stanford-Binet Intelligence Scale 4th Edition (S-BIS IV) administered at three and five years of age. Covariates investigated included maternal IQ, parity (number of births), age at delivery, household income, prenatal smoking, race and education; and subject birth weight, gestational age at birth, iron status, HOME scale score at 24 months and HOME Short Form (HOME-SF) at six years, and crowding in the home. Exclusion criteria included low birth weight, preterm birth, English as a second language and Down's Syndrome.

Blood lead, whether expressed as concurrent, lifetime average, average in infancy (6-24 months) or peak blood was significantly and negatively associated with decrements in adjusted IQ scores at three and five years of age. Similar results, but a greater effect size (steeper dose-response), was reported when the analysis was restricted to subjects whose peak blood lead never exceeded 10 µg/dL. Analysis with polynomial (cubic and quadratic) and nonparametric models provide evidence

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of nonlinearity in the lead-IQ association. A penalized-spline model indicates an IQ decrement of 7.4 points for an increase of lifetime average blood lead from 1 to 10 μ g/dL. No confidence intervals were calculated for this semiparametric estimate.

The Canfield *et al.* (2003a) publication is noteworthy for at least two reasons: (1) It confirmed lead effects on neurocognitive development at blood lead concentrations less than 10 μ g/dL that had earlier been reported in cross-sectional studies (Chiodo *et al.*, 2004; Fulton *et al.*, 1987; Lanphear *et al.*, 2000); and (2) It drew attention to the apparently curvilinear dose-response relationship between lead and IQ effects. The authors reported over a two-fold increase in effect size for children whose blood lead never exceeded 10 μ g/dL relative to children whose peak blood lead had exceeded 10 μ g/dL.

Performance on colour identification and task completion of the Shape School Task at four and five years of age and neuropsychological tests of executive processing at five and a half years of age were also significantly negatively associated with blood lead concentrations among this cohort (Canfield *et al.*, 2003b; Canfield *et al.*, 2004), providing additional evidence that the neurodevelopmental effects of lead exposure may not have been limited to global measures of intellectual functioning.

A follow-up study on the association between lead exposure and IQ at six years of age among the Rochester cohort was reported by Jusko *et al.* (2008). Psychometric intelligence was measured in 174 subjects using the Wechsler Preschool and Primary Scale of Intelligence, Revised (WPPSI-R). The authors reported that they used the WPPSI-R because it provides a better assessment of children with below average IQ scores. Four blood lead exposure variables were analyzed: Lifetime average, infancy (6-24 months) average, peak and concurrent. Covariates investigated included maternal IQ, parity, age at delivery, household income, prenatal smoking, race and education; and subject birth weight, gestational age at

birth, iron status, HOME scale score at 24 months and HOME Short Form (HOME-SF) at 6 years, and crowding in the home. Low birth weight, preterm birth, English as a second language and Down syndrome were exclusion criteria.

Two analytical strategies were used to assess the relationship between blood lead and IQ. First, blood lead was modeled categorically: In this analysis, adjusted IQ was statistically lower for those subjects with a lifetime average, infancy average, and peak blood lead of 5-9.9 µg/dL than for those subjects with these measures of blood lead < 5 μ g/dL. For example; the adjusted IQ of children with lifetime average blood lead of 5-9.9 µg/dL was approximately five points lower than subjects with a lifetime average blood lead of $< 5 \mu g/dL$. In a second analysis, a generalized additive model (GAM) with locally weighted scatterplot smoothing (Loess) was used to generate a semiparametric model of the relationship between continuous peak blood lead (excluding the top 3% of values) and IQ. This analysis revealed a statistically significant non-linear inverse association between peak blood lead and adjusted IQ. This relationship was evident down to the lowest measured peak blood lead value (2.1 µg/dL). There was also a steeper dose-response observed over the lower range of peak blood lead values. Adjusted IQ decreased by approximately 1.2 IQ points per 1 μ g/dL blood lead over the range of 2.1-10 μ g/dL – the slope of this doseresponse relationship was approximately an order of magnitude lower over the range of 20-30 µg/dL.

Shanghai, China

Shen *et al.* (1998) reported on the longitudinal association between prenatal and postnatal blood lead and infant neurological development among 133 urban infants living in Shanghai, China. Lead was measured in cord blood and capillary blood at three, six, and 12 months of age. Neurodevelopmental effects were assessed with the Mental Development Index (MDI) and the Psychomotor Development Index

(PDI) of a translated and locally standardized version of the Bayley Scales of Infant Development. The influence of a wide range of potential medical, demographic and social and environmental covariates was investigated, but these did not include maternal IQ, HOME environment or similar, nor nutritional status of the subjects. Because of the importance of these potential confounders the results of Shen *et al.* (1998) are given reduced weighting in this report. Analysis of covariance (ANCOVA) was used to determine significant differences in adjusted outcome between the high cord blood lead tertile (11-18 μ g/dL) and the low cord blood lead tertile (2-7 μ g/dL). Adjusted MDI scores were significantly negatively associated with cord blood lead at all three test periods. No significant association between cord lead and PDI was reported, however the authors noted that the PDI was reportedly a less sensitive subscale. Infant MDI and PDI were not associated with prior or concurrent postnatal blood lead at any of the three follow-up periods.

Sydney, Australia

A cohort of 318 middle class, mixed sex, Caucasian mother-infant pairs were recruited from three hospitals in Sydney, Australia from 1982-83 and followed for seven years. Lead exposure amongst this cohort was relatively low (80% of the cord blood lead concentrations were $\leq 10 \ \mu g/dL$) and maternal IQ and child performance on psychometric tests of intelligence and development was relatively high. Blood lead was sampled at birth (maternal blood lead and cord lead) and postnataly at 6 month intervals until four years of age and then again at five years. All children's blood samples collected after two years and maternal blood samples were venous. Children's blood samples at 6, 12, 18 and 24 months were collected via capillary samples. Cooney *et al.* (1991) reported that up to 5% of the capillary samples may have been contaminated.

Neurological development and functioning was assessed at 6, 12 and 24 months with the Bayley Scales of Infant Development, at three and four years with the McCarthy Scales of Children's Abilities, and the Wechsler Intelligence Scale for Children-Revised (WISC-R) at age seven.

Measured covariates included maternal age at birth, verbal IQ, education, occupation and smoking and alcohol consumption during pregnancy; father's age, education, and occupation; quality of the caregiving environment (HOME score measured annually) and subject gestational age, birthweight, and a 43 item obstetrical complications checklist. Exclusion criteria included children of single mothers, non-English speaking mothers, mothers with drug or alcohol problems, and babies with severe medical conditions, <37 weeks gestation, or birth weight <2,500 g.

Cooney *et al.* (1989b) reported on the observed relationships between maternal and cord blood lead and measures of neurological development up to the age of 3. Associations between maternal or cord blood lead and serial measures of neurological development were explored using multiple regression analysis. No significant negative associations between lead and neurological outcomes were reported. An additional longitudinal analysis of the potential relationships between maternal or cord blood lead and changes in neurological development over time was conducted using path analysis based on analysis of covariance structures. This analysis was conducted to explore the question of how much of a subject's change in neurological development over time can be attributed to prenatal lead exposure (as measured by maternal blood lead and changes in neurological development over time trelationships between blood lead and changes in neurological development over time was conducted to explore the additional lead exposure (as measured by maternal blood lead and changes in neurological development over time trelationships between blood lead and changes in neurological development over time was conducted blood lead and changes in neurological development over time trelationships between blood lead and changes in neurological development over time was conducted blood lead and changes in neurological development over time trelationships between blood lead and changes in neurological development over time was relationships between blood lead and changes in neurological development over time was related blood lead and changes in neurological development over time was related blood lead and changes in neurological development over time was related blood lead and changes in neurological development over time was related blood lead and changes in neurological development over time was related blood lead and changes in neurological development over time was related blood lead and changes in neurological development over time was related

The multiple regression models developed in the analysis of the Sydney cohort data were not able to explain as much of the variance in the dependent variable as was achieved in other studies (Ernhart *et al.*, 1987; Wasserman *et al.*, 1994). The relatively greater signal to noise ratio in the Sydney data reduces the ability to detect an effect of lead.

Cooney *et al.* (1989a) reported on the results of the Sydney study up to age four for 207 subjects. Only six covariates were used in the regression models at this period of analysis; the authors report that these covariates were selected based on results of previous analyses. Three statistical strategies were employed in this paper: (1) multiple linear regression of the association between annual average blood lead and MSCA GCI at age four; (2) analysis of covariance of the relationship between current and previous year's blood lead and change in developmental outcome from 3 to 4 years; and (3) the association between a measure of cumulative blood lead and the developmental outcome at age four. None of these analyses, after adjusting for covariates, produced a significant relationship between blood lead and MSCA GCI at age four.

Cooney *et al.* (1991) reported on the results of potential associations between lead and IQ (WISC-R) among 175 children from the Sydney cohort at age seven. Multiple linear regression was used to test for cross-sectional associations between IQ at age seven and serial measures of lead exposure at various ages up to seven. The same six covariates as those selectedby Cooney *et al.* (1989a) were used in the regression analysis at age seven. No significant associations between blood lead and IQ at age seven were reported. An additional, longitudinal analysis was also employed to test the effect of lead on the rate of change of neurological development. Again, no significant association was reported.

Treatment of Lead Exposed Children (TLC) Trial

The Treatment of Lead Exposed Children (TLC) was a multi-center (Philadelphia, PA; Newark, NJ; Cincinnati, OH; and Baltimore, MD), randomized, placebocontrolled, double-blind clinical trial designed to assess the effects of the oral chelating drug succimer⁶ on the cognitive, behavioural and physical development of moderately lead-exposed children (blood lead at enrolment was 20-44 µg/dL). Up to three 26-day courses of succimer or placebo therapy were administered depending on response to treatment. While succimer treatment did transiently lower blood lead for about 10 months, it did not result in any improvement in performance on tests of behavioural and psychological function at 36 (Rogan *et al.*, 2001) and 60 months (Dietrich *et al.*, 2004).

Seven hundred and eighty mixed sex, predominantly African-American, lower socioeconomic status subjects were enrolled at about two years of age and followed until seven years of age. Blood lead was quantified frequently: Days 7, 28 and 42 of treatment and every 3 to 4 months post treatment. Various psychometric tests were administered at different times: the Bayley Scales of Infant Development-II (BSID-II) at baseline, the WPPSI-R at 36 month follow-up and, the WISC-III at 60 month follow-up. Covariates included clinical center; subject age, race, sex, language; caregiver IQ; and parental education, employment, and marital status. No data was taken on the quality of the care giving environment – a potentially important confounder (Greene *et al.*, 1992).

Chen *et al.* (2005) used multiple linear regression models to analyze the crosssectional associations between blood lead and psychometric test scores at various ages. Prior blood lead was included in the models as an independent variable to examine the potential residual effects of earlier blood lead concentrations. A binary

⁶ Chemical name: *meso* 2, 3-dimercaptosuccinic acid (DMSA); Brand name: Chemet

variable was constructed for this parameter to minimize the effects of collinearity between consecutive blood lead measurements.

A significant negative inverse association between blood lead and covariate adjusted psychometric test scores was reported for all measures of blood lead (peak, average and concurrent), except for IQ at seven years of age and blood lead at two years and peak blood lead to seven years of age. Adjustment for prior IQ did not significantly affect the results. The association between blood lead concentration and IQ score was homogenous between treatment groups except for baseline blood lead and baseline BSID-II, where a steeper dose-response relationship was reported for the placebo group. Of the various blood lead measures, concurrent blood lead always had the strongest association with the psychometric test scores and these relationships became stronger (greater effect size) as the subjects got older. The results are illustrated in Figure 5. The results of the Chen *et al.* (2005) study suggest that: (1) cross-sectional associations between IQ and blood lead observed in older children are not only attributable to residual effects from higher, unmeasured blood lead concentrations earlier in life; and (2) six to seven year old children may be as or more sensitive to the neurological effects of lead exposure as two year-old children.



Figure 5. Reproduced from Chen et al. (2005). IQ test scores at 2 years, 5 years and 7 years by prior to concurrent blood lead concentration. Data are grouped by blood lead quartile. The Y value corresponds to the mean test score of the group; the x value corresponds to the median blood lead of each quartile.

Yugoslavia Prospective Study of Environmental Lead Exposure

Subjects for the Yugoslavia Prospective Study of Environmental Lead Exposure were recruited from two towns in Kosovo, Yugoslavia: Kosovska Mitrovica (Mitrovica) is host to a lead refinery, smelter, and battery factory; Pristina is a town 25 miles to the south with no significant point sources of lead exposure (lead paint was banned in Yugoslavia in 1922 and automobile traffic was minimal). Because of this unique approach to subject recruitment, the Yugoslavia study has the widest range of lead exposure (children's blood lead ranged from 1-70 µg/dL) and socioeconomic status of all the prospective lead studies. Seven hundred and six mother-infant pairs were recruited in 1985 and 1986. Assessment of health outcomes predated the hostilities between Serbia and Kosovo. Interview and assessment instruments were translated into Serbo-Croatian and Albanian.

Maternal blood lead was sampled at mid-pregnancy and cord blood was sampled at delivery. Postnatal blood lead (venous) was sampled semi-annually from birth until 7.5 years of age and then again at about 10 years of age. All measures of blood lead were highly correlated. Correlations among prenatal, early postnatal and latter postnatal ranged from 0.90 to 0.95. Tibia lead was measured *in* vivo by K-XRF at 11-13 years.

A wide range of medical, biological, socioeconomic and environmental covariates were examined, including nutritional status, maternal intelligence and quality of the care-giving environment (HOME score assessed at age 3-4 and 9-10). Socioeconomic status was not directly measured, but ethnicity (Albanian, Serbian or other) was thought to provide a reasonable surrogate for SES. Exclusion criteria included gestation <28 weeks or >44 weeks, multiple births, residence more than 10 km from the community pediatric center, and those with chromosomal abnormalities or nervous system defects.

Children were assessed on the Bayley Scales of Infant Development (BSID) at 6, 12, 18 and 24 months and the McCarthy Scales of Children's Abilities (MSCA) at age three and four. Intelligence was assessed at age five and again at about age seven using the Wechsler Preschool and Primary Scale of Intelligence-Revised (WPPSI-R) and at age 10 to 12 using the Wechsler Intelligence Scale for Children-Version III (WISC-III).

Wasserman *et al.* (1992) reported on the cross-sectional associations between BSID MDI scores of 392 children at two years of age and cord blood lead and infant blood lead at 6, 12, 18 and 24 months. Inverse associations were reported for all blood lead measures, but only the 24 month blood lead was statistically significant after adjusting for covariates. Wasserman *et al.* (1992) also reported an association between decreased haemoglobin at 18 months and poorer performance on the BSID MDI at 24 months.

Wasserman *et al.* (1994) reported on the results of the neurodevelopmental assessments at ages three (388 children) and four (332 children). MSCA General Cognitive Index (GCI) test scores at age four were, after adjusting for covariates, significantly and inversely associated with prenatal, cord and postnatal (eight serial, semi-annual measures) blood lead. The authors report similar results for MSCA scores at age three, but the analysis is not presented.

The relationship between lead exposure and children's intelligence measured at 5 years (318) and from 6.5 to7.5 years (309) was analyzed by Wasserman *et al.*(1997). Lead exposure was expressed as the area under the blood lead curve (AUC - trapezoidal approximation) from birth until age of assessment (5 and 7 years). After adjusting for covariates blood lead AUC from birth to seven years and blood lead AUC from birth to five years was significantly and negatively associated with IQ.

In the Yugoslavia cohort, higher lead exposure was associated with higher SES. A similar situation also occurred in the Boston study cohort. In studies from both of these cohorts, the lead-IQ relationship was strengthened after correcting for covariates. This is opposite to the effect of adjusting for covariates in other studies where lead exposures are inversely related to SES. This demonstrates that, if the observed association between blood lead and IQ is a product of uncontrolled confounding, the confounding variable is unrelated to SES.

Wasserman et al. (1997) conducted a longitudinal analysis using 1167 observations from 390 children from the Kosovo cohort who were assessed for intellectual functioning at three, four, five or seven years of age. This analysis used a generalized estimating equation (GEE) repeated-measures approach that takes into account the within subject correlation in the repeated measures of outcome. This allows for an increase in the number of observations and a resulting increase in statistical power. In their analysis, Wasserman et al. (2000a) examined the influence of relative patterns of change in postnatal blood lead and IQ while controlling for prenatal blood lead. Estimates of effect, therefore, were based both on the subject's absolute prenatal blood lead and on their pattern of change in postnatal blood lead defined as a function of their prenatal blood lead (> or < 50% change). Both prenatal blood lead (average of mid pregnancy and cord blood lead) and a pattern of increased postnatal blood lead (>50% increase over prenatal blood lead) were significantly negatively associated with IQ after adjusting for confounders. This analysis suggests that it is the proportional change in postnatal blood lead, relative to prenatal blood lead concentrations, that is the important determinant of lead effects on IQ, rather than the absolute value of postnatal blood lead.

Examining the relationship between serial blood lead and outcomes one at a time, rather than controlling for one time period while examining relationships in other

periods, can lead to confounded relationships where there is significant correlation amongst serial measures of blood lead.

Wasserman *et al.* (2000a) further examined the relationship between intelligence assessed at ages 10 to 12 (WISC-III) and both blood (290 subjects) and tibia lead (167 subjects). Tibia lead was quantified by K-XRF at ages 11 to 13. Repeated measures linear models (generalized estimating equations) were used to account for within subject correlations of repeated blood lead measures. In addition to the standard covariates, concurrent blood lead and anthropometric measures (height, weight, shoulder width, tibia width and length, calf circumference and skin fold thickness) were considered in the tibia lead regression analysis. After adjusting for covariates, average blood lead to age 10 and concurrent blood lead were both significantly and inversely associated with IQ at age 10 to 12. However, after tibia lead was entered into the model, neither measures of blood lead retained significance, but tibia lead demonstrated a significant inverse relationship with IQ. This suggests that, for school-aged children, associations between bone lead and IQ are stronger than those between blood lead and IQ.

As a child ages, blood lead is more strongly influenced by cumulative exposure (i.e. greater relative contribution from endogenous sources). Wasserman *et al.* (2003) reported increasing correlation coefficients between concurrent blood lead and cumulative blood lead index (CBLI) with increasing age. A greater correlation was reported for higher lead exposure.

International Pooled Analysis

Lanphear *et al.* (2005) reported on a pooled analysis of data from seven of the eight longitudinal prospective studies that were initiated prior to 1995 and followed subjects until at least five years old. The analysis involved 1,333 children with complete data on requisite covariates. The participating studies included Boston,

MA; Cincinnati, OH; Cleveland, OH; Rochester, NY; Mexico City; Port Pirie, Australia; and Kosovo, Yugoslavia. Data from the Sydney, Australia cohort was not included in the pooled analysis. It should be noted that US EPA (2007a) has posted a correction to the Lanphear *et al.* (2005) publication and attributed the corrected data to personal communications with Dr. Lanphear. The data presented in the US EPA (2007a) memo was used in this report.

Full scale IQ was the outcome assessed. Subject's IQ was assessed at primary school age (mean age of assessment 6.9 years; range 4.8-10 years; five of the seven studies assessed IQ at six or seven years old) with an age and language appropriate version of the Wechsler Intelligence Scales for Children (WISC-R, WISC-III, WPPSI, or WISC-Spanish).

Children's blood lead was sampled by venous or capillary sample, depending on the protocols of the individual participating studies (see above). Four blood lead indices were used in the analysis: (1) concurrent; (2) maximum; (3) lifetime average; and (4) early childhood, defined as the six month to two year mean blood lead. Data on cord blood lead was also available for 696 subjects. The pooled median concurrent, maximum, lifetime average and early childhood blood lead was 9.7 μ g/dL, 18.0 μ g/dL, 12.4 μ g/dL, and 12.7 μ g/dL, respectively. One hundred and three (8%) of subjects had a maximum blood lead < 7.5 μ g/dL.

Data on the following covariates were included in the pooled analysis: maternal IQ, education, marital status and prenatal alcohol and tobacco use; HOME Inventory score; and subject sex, birth order, and birth weight. The influence of ethnicity was investigated for the sub-set of US data. Potentially important covariates that were not included in the pooled analysis include SES and nutritional status.

Multiple fixed effect regression modeling was used to investigate the associations between blood lead indices and IQ. In a comparative analysis of the respective coefficients of determination of the linear regression models, concurrent blood lead was identified as the blood lead index that explained the greatest variance in IQ.

Quadratic and cubic terms were added to the adjusted linear regression models to test for linearity. Both the quadric and cubic terms were statistically significant and a restricted cubic spline model was used to illustrate the shape of the blood leadresponse relationship. A log-linear model was identified as the parametric function that most closely matched the shape of the blood lead-response relationship indicated by the restricted cubic spline model.

After adjusting for covariates, significant inverse associations were reported between all four blood lead indices and full scale IQ. The authors report a decline of 6.2 points in full scale IQ for an increase in concurrent blood lead levels from <1 to 10 µg/dL. A significant inverse association was also reported for concurrent blood lead and performance and verbal IQ. Ethnicity did not affect the concurrent blood lead-IQ relationship for the subset of subjects from the US studies where data on this variable was available. The inverse relationship between cord lead and IQ was not statistically significant after adjusting for covariates.

Linear regression models were also constructed for various maximum blood lead cut-points that had been identified *a priori*. The adjusted linear regression coefficient between concurrent blood lead and IQ for those subjects with a maximum blood lead < 7 μ g/dL was less than and significantly different from those subjects with a maximum blood lead > 7 μ g/dL. The magnitude of difference in the slopes of the blood lead-response relationship was about 20 fold. The difference in blood leadresponse slopes for those subjects with a maximum blood lead of < 10 μ g/dL was not significantly different from those subjects with a maximum blood lead of > 10 μ g/dL. This analysis provides additional strength to the evidence that the blood lead-IQ relationship becomes shallower at blood lead concentrations greater than about 7-10 μ g/dL.

Sensitivity analysis demonstrated that the results of the pooled analysis were not driven by any single particular study cohort or the use of a fixed effects rather than random effects model.

Summary of Longitudinal Studies

As of January 1st 2008 there were 12 published longitudinal studies of early life lead exposure and potential effects on psychometric test scores and also a pooled analysis of the results of seven of the eight of these studies that had been initiated prior to 1995. In summary, this body of evidence is strongly suggestive, but not consistently supportive, of an association between early life chronic lead exposure (as measured by various biomarkers) and decrements in school-aged children's IQ, after correcting for covariates. This association has been demonstrated in a wide variety of ethnic and socioeconomic populations across a wide range of lead exposures. Four of the 12 longitudinal studies and the international pooled analysis are strongly supportive of an association between early life lead exposure and potential effects on psychometric test scores. Six of the 12 longitudinal studies report inconsistent results across biomarker of lead exposure, time point of exposure or assessment, or neurological assessment tool. Two of the 12 longitudinal studies are strongly negative. The pattern of results does not appear to be dependent on cohort demographics, such as SES, nor do they appear to be dependent on exposure range – significant associations have been reported among both relatively low and relatively high socioeconomic strata as well as across relatively low and relatively high blood lead concentrations. One pattern to the results of the longitudinal studies is that the three studies with the most subjects were all strongly positive. These were the TLC Trial, Port Pirie and Kosovo cohorts. The results of the international pooled

analysis are also consistent with this pattern. The results of the analysis of data from a combined 1,303 subjects were consistently and strongly positive for a significant inverse association between children's blood lead concentrations and corrected IQ. The one exception to this pattern is the results from the Rochester cohort, which were also consistently and strongly positive, but were observed in analysis with relatively few (~170) subjects.

In general, findings between neonatal and infant neurodevelopment and postnatal blood lead concentrations are equivocal or perhaps leaning towards negative. There is stronger and more consistence evidence of an association between neonatal, infant and toddler neurodevelopment and maternal lead (blood and bone) and cord blood. Several challenges exist that limit the ability of investigators to elucidate a relationship between blood lead concentrations and neurological effects in early life. Neurocognitive abilities become more stable, less variable, and better standardized as children become older. Changes in blood volume and physiology can potentially influence blood lead concentrations in the neonate. These factors introduce more uncertainty into both measures of effect and measures of exposure – making null findings more likely.

3.2.2 Supporting Epidemiological Evidence from Meta-Analysis and Cross-Sectional Studies

The following section presents a review of some of the additional relevant epidemiological literature on the association between early life lead exposure and IQ decrements in school-aged children. Included in this review are recent metaanalyses as well as publications that explicitly sought to examine the shape or extent of the biomarker-response relationship between blood lead and children's IQ.
Meta-Analyses

Recent meta-analyses are presented as evidence supporting the results of the international pooled analysis. Like the pooled analysis, the meta-analyses integrate data from multiple studies and provide another tool for summarizing the combined results of multiple studies with varied results. However, in contrast to the pooled analysis, the meta-analyses include data from cross-sectional studies, which may be more susceptible to exposure misclassification. The inclusion of cross-sectional blood lead data also raises the issue of temporality of exposure and effects (this is less of a concern for dentin lead, which is a better biomarker of cumulative exposure). For these reasons, the results of the meta-analyses are accorded less weight than those of the pooled analysis.

A number of meta-analyses and attempted meta-analyses have been published on the association between blood lead and children's IQ. Because of methodological limitations with earlier studies, such as inconsistencies in controlling for confounding variables, as well as the higher range of blood lead values examined in these studies, only meta-analyses published since 1990 are considered in this report.

Four meta-analyses published since 1990 were identified and reviewed for this report. They are summarized below. The meta-analyses were unanimous in their conclusions that the available epidemiological evidence supports an inverse association between childhood blood lead and IQ over the range of lead exposures captured by the constituent studies. The quantitative effect size of two of the meta-analyses is similar, but this is not surprising given that they drew from the same pool of available literature. The quantitative effect sizes reported by these meta-analyses are not emphasized here nor were they considered candidate critical studies for the TRV derivation because the blood lead-IQ relationship reported for older (and higher exposure) studies may not be representative of the blood lead-IQ relationship associated with contemporary levels of environmental lead exposure. The meta-

analyses that are discussed in more detail below include Schwartz (1994a), Pocock *et al.* (1994), Needleman *et al.* (1990), and Thacker *et al.* (1992).

Schwartz (1994a) used inverse variance weighting and a random effects model to conduct a meta-analysis on three longitudinal and four cross-sectional studies of blood lead and full scale IQ in school age children. The results of this analysis must be treated with caution because the authors provide little detail on study selection criteria and not all available studies were included in the analysis. The effect size was expressed as the change in IQ per doubling of blood lead from 10 to 20 µg/dL. For longitudinal studies, blood lead measured at two years or the closest available estimate was selected as the exposure estimate. Based on a meta-analysis of seven studies, Schwartz (1994a) report an estimated full scale IQ decrement of 2.57 (SE 0.41) IQ points as blood lead increases from 10 to 20 µg/dL. The authors did not report the combined *n* or *p* values. Diagnostics demonstrated that this estimate was not overly sensitive to any one study and that even if combined with eight hypothetical additional studies that showed a magnitude of effect of zero, there would remain a statistically significant negative association – although the magnitude of the effect size would be reduced by a factor of 50%. The magnitude of effect of the combined longitudinal studies was only slightly higher (2.96 ±1.25) than that of the cross-sectional studies (2.69 ±0.51). Stratification by socio-economic status of the study cohorts demonstrated that a stronger effect size was reported for higher socio-economic status cohorts (2.89 ± 0.50) than that of lower socio-economic status cohorts (1.85 ±0.92). Studies among the lower SES cohorts had a lower effect size and greater variance.

Pocock *et al.* (1994) conducted a systemic review and meta-analysis of studies of children's blood or dentine lead and IQ in school-aged children published in the period from 1979 to 1993. The review included 26 studies: 5 prospective longitudinal studies of blood lead and IQ, 14 cross-sectional studies of blood lead and IQ, and

seven (cross-sectional) studies of dentine lead (whole tooth) and IQ. The authors concluded that a doubling of blood lead (from 10 μ g/dL to 20 μ g/dL) or dentine lead (from 5 to 10 μ g/g) would result in an estimated decrement in full scale school-aged IQ of 1-2 points. The range of exposures for which the effect size was estimated were selected to be representative of the ranges of exposures examined in the individual studies. A fixed effect model was used to estimate the effect size for the combined studies.

The meta-analysis of Pocock *et al.* (1994) quantified the relationship between lead and IQ in the following the ways: (1) from the five prospective studies, the associations between perinatal blood lead, the best estimate of two year blood lead and postnatal mean blood lead and school age IQ (range 5 to 10 yrs); (2) from the 14 cross-sectional studies, concurrent blood lead and school age IQ (range 5.5-12 yrs); (3) from the 7 studies of tooth lead, tooth lead (location not specified) and school age IQ (range 6-9.4 yrs).

The Pocock *et al.* (1994) meta-analysis of cross-sectional blood lead and dentine lead did show a significant negative association with IQ, but there was significant heterogeneity among the subject studies and the meta-analysis included study results that were not adequately corrected for confounding variables. The crosssectional blood lead studies possessed the least heterogeneity in terms of controlling for confounding and estimates of lead exposure. The results of the metaanalysis using an inverse variance fixed effect model report no significant association between prenatal nor perinatal mean blood lead and IQ, but a significant negative association was reported for the best estimate of two year old blood lead and IQ. The magnitude of the effect from the meta-analysis was -1.85 (standard error (SE) 0.51) IQ points per doubling of two year old blood lead from 10 to 20 µg/dL. The authors noted that the study weighting associated with this estimate of effect size appeared anomalous and that this casted doubt on the validity of the estimate.

Thirty five reports from five longitudinal studies of children's blood lead and IQ were assessed for quality by a blinded review panel (Thacker *et al.*, 1992). The panel concluded that a quantitative estimate of the combined studies would be inappropriate due to inconsistencies in methods of analyzing and reporting data.

Needleman *et al.* (1990) conducted a meta-analysis of seven blood lead and five dentin lead studies. The 12 studies included in the meta-analysis were drawn from a pool of 24 studies on the subject published between 1972 and 1989. The combined blood lead-IQ association was not significant, while the combined dentin lead-IQ relationship was inverse and significant.

Overall, the results of the four meta-analyses on children's blood, and in some cases dentin, lead provide supportive evidence of an inverse association between children's lead exposure and IQ. Important limitations include the previously mentioned methodological shortcomings of some of the individual subject studies and questions about whether the quantitative biomarker-response relationship generated by these studies can be considered representative of those potentially associated with contemporary environmental lead exposures and associated blood and dentin lead concentrations.

3.2.3 Bone lead

Only one study that examined the relationship between bone lead and IQ in children was identified at the time of this report - that from the Yugoslavia cohort (Wasserman *et al.*, 2003). Bellinger *et al.* (1994a) examined the relationship between tibia and patella lead and neuropsychological tests of attention (Mirsky

battery) among 79 young adults (19-20 years old). Subjects had participated in Needleman's original study of dentin lead and neurological outcomes (Needleman *et al.*, 1979). Tibia lead concentrations were inversely associated with covariate adjusted scores on tests of focus and execution and positively associated with adjusted errors on the L'Anthony Hue Test (colour discrimination). No other significant associations were reported. Needleman *et al.* (1996) also reported a significant association between tibia lead measured at 12 years and antisocial and aggressive behaviour as rated by teacher and parents on the Child Behaviour Checklist (CBCL). Given the paucity of epidemiological evidence on the relationship between bone lead and IQ or other reliable measures of neurological development or functioning, no bone lead based TRV can be derived for early life lead exposure and developmental neurotoxicity.

3.2.4 Susceptible Life-Stages

The developmental neurotoxic effects of lead exposure have been measured in longitudinal studies out to at least 17 years old. For example, Ris *et al.* (2004) demonstrated that blood lead concentrations measured in subjects of the Cincinnati Lead Study (CLS) at six and a half years old were related to deficits in IQ, academic achievement and other dimensions of neurological functioning (attention, visio-construction, and fine-motor skills) at 15 to 17 years of age. Significant inverse associations between early life blood lead and covariate adjusted IQ were also reported to at least 10 years of age among subjects of the Mexico City Prospective Lead Study (Schnaas *et al.*, 2000), Yugoslavia Prospective Lead Study (Wasserman *et al.*, 2003), Port Pirie, Australia cohort (Tong *et al.*, 1996), and Boston, USA cohorts (Bellinger *et al.*, 1992). Needleman *et al.* (1990) also reported an association between dentin lead levels in deciduous teeth shed at six to seven years old with impaired neurological and behavioural effects in late teens. Therefore, the endpoint is explicitly defined as IQ deficits in school-aged children (i.e. up to age 18).

However, it should be noted that there is no evidence that decrements in school-age IQ are reversible. Additionally, academic, behavioural and social challenges manifested in school-aged children have potentially life-long health and socioeconomic implications.

The results of studies of prenatal lead exposure and developmental neurotoxicity have not been as consistent as those examining postnatal blood lead and school-aged IQ deficits. However, both exposure and outcome are more difficult to measure for the prenatal blood lead and early neurological development relationship that has most often been the subject of investigation. Maternal blood lead varies throughout the time course of pregnancy. Maternal blood lead collected at one time period or a cord blood sample may give an imprecise measure of the levels of lead exposure experienced by the developing fetus throughout gestation. Psychometric tests of intelligence or neurological development that are administered in infancy or in preschool years are less stable (i.e. tend to vary more over time) and have less validity than tests administered at older ages (Ross, 1989). The combined effects of greater imprecision in measuring both exposure and effect could be potentially null biasing and may have contributed to the greater inconsistency in results among epidemiological studies of the maternal blood lead-response relationship for this endpoint.

Despite the potential difficulties with measuring prenatal lead exposure and early life neurological development, there are some notable positive study results that deserve emphasis. Schnaas *et al.* (2006) reported a significant inverse relationship between gestational week 28 maternal blood lead and covariate adjusted IQ measured annually from 6 to 10 years of age among 150 subjects of in the Mexico City Prospective Lead Study. Maternal blood lead was sampled every 8 weeks starting at week 12 of gestation and this study was able to quantify maternal blood lead levels over the course of gestation better than any other of the available

longitudinal studies. At the time of this report, Gomaa *et al.* (2002), is the only publication that reported on the relationship of maternal bone lead and developmental neurotoxicity, also reported a significant inverse relationship between maternal patella lead and infant neurological development as measured by the BSID at age two.

While the evidence of an association between prenatal lead exposure (as measured by maternal blood or bone lead or cord lead) and school-aged IQ decrements is not as strong as the evidence linking postnatal children's blood lead and school-aged IQ, there is insufficient evidence or logic reasons to reach the conclusion that the fetus would be less vulnerable than a child to the developmental neurotoxic effects of lead. Therefore, it is recommend that the TRV for this endpoint should also be applied to prenatal exposures and, by extension, to women of childbearing age.

3.2.5 Unmeasured Confounding

Some commentators have argued that the observed relationships between early life lead exposure and developmental neurotoxic effects are an artefact of unmeasured or residual confounding. This argument is inconsistent with the available evidence. Firstly, as will be reviewed below, there is strong evidence of the developmental neurotoxicity of lead from *in vivo* animal experiments and there are plausible mechanisms for these effects in humans. Secondly, a significant inverse association between biomarkers of early life lead exposure and covariate adjusted scores on tests of psychometric intelligence and other neurologic endpoints have been observed in a diversity of cohorts with varied socioeconomic and ethnic backgrounds and with a diversity of dominant lead exposure sources. If the observed adverse effects are attributable to unmeasured confounding, the confounding variables must be unrelated to socioeconomic status, ethnicity, or lead source. For example, similar effect size was observed for both the Boston and Rochester cohorts - two cohorts

with very different socioeconomic status (SES). This suggests that if the observed biomarker-response relationship is due to residual confounding, it is unlikely that the unmeasured confounding is related to SES (Canfield *et al.*, 2003a). Also, for both the Boston and Yugoslavia cohorts, the effect size of lead on IQ increased after adjusting for the potential influence of SES. Overall, there is sufficient evidence from the collection of observational studies that have done a reasonable job of investigating the influence of potentially confounding variables that the reported effect is unlikely entirely attributable to unmeasured confounding. While it is agreed that some of the effect may be due to unmeasured confounding, it is also equally likely that the reported effect sizes have been influenced (reduced) by over-control of potential confounders (Bellinger, 2004).

3.2.6 Summary of Epidemiological Evidence

On balance, the longitudinal epidemiological evidence is strongly suggestive, but not consistently supportive, of an association between early life chronic lead exposure and decrements in school-aged children's IQ. When weighed in the context of the strong supporting evidence from cross-sectional epidemiological studies and *in vivo* and *in vitro* lines of evidence (summarized below), there is sufficient evidence that lead is a developmental neurotoxin and that this endpoint can and should be used as a critical effect for the derivation of a TRV for lead. There is no biological or mechanistic reason to reject a curvilinear biomarker-response relationship and there is strong evidence of a steeper blood lead-IQ relationship over the lower range of blood lead concentrations examined in the existing studies – a range that includes contemporary blood lead concentrations among environmentally exposed Canadians. There is little evidence of a threshold for these observed effects. Therefore, it is recommended that the exposure- response relationship for the developmental neurotoxic effects of early life lead exposure be extended at least as

low as the lower ranges of biomarker concentrations that have been studied in the existing literature (i.e. at least as low as 0.5–1.0 ug/dL).

3.3 EVIDENCE OF DEVELOPMENTAL NEUROTOXICITY FROM ANIMAL EXPERIMENTS

Introduction

The following section presents an overview of the *in vivo* experimental evidence of the neurotoxic effects of lead exposure. The purpose of this section is to weigh the *in vivo* evidence to determine if it supports the observed epidemiological associations between early life lead exposure and decrements in the IQ of school-aged children.

There is no equivalent endpoint to human psychometric tests of intelligence (IQ) in animal studies. Experimental animal studies, instead, rely on behavioural tests of cognitive function. These experiments compare the performance of exposed animals to controls in the learning and performance of a reward- motivated behaviour. This usually necessitates the requirement for repeat testing, which can make the statistical analysis and interpretation of behavioural animal assays relatively complex. In this respect, the relative changes in performance over time provide an added dimension to the dose-response relationship. Additionally, as will be explained below, the history of prior training and testing among the test animals can also influence assay results.

Lead has been shown to produce neurochemical, neurophysiological, structural, and behavioural neurotoxic effects. The focus of this review, however, is on behavioural functional effects. Neurophysiological and neurochemical functional effects and structural effects are not provided the same emphasis and depth of review because the relationship between these effects and the critical effect (decrements on psychological tests of human intelligence) is not as direct as some of the reported behavioural functional effects of lead exposure *in vivo*. Behavioural effects are also an integrated measure of other functional and structural neurotoxic effects.

Many of the *in vivo* behavioural effects of lead have been demonstrated through assessment of operant conditioning or schedule-controlled operant behaviour Operant conditioning is a type of conditioning where behaviour is learned through a system of rewards for "correct" behaviour. The correct behaviour can include such tasks as waiting for a specified time interval (Fixed Interval), pressing a lever a specified number of times (Fixed Ratio), or responding to visual or auditory cues. Operant conditioning can test many domains of cognitive functioning, including learning, memory, attention span (distractibility), and the ability to inhibit inappropriate behaviours.

As mentioned, the scope of this section is primarily limited to a review of the *in vivo* evidence of lead-induced impairments in performance on neurobehavioural tests of cognitive function. There is strong evidence of CNS damage in lead exposed animals from a wide variety of related endpoints, including biochemical markers of effect, pathological disturbances, and functional impairments. Animal studies demonstrate lead-induced adverse effects on auditory function (Rice, 1997); visual structure and function (Rice, 1998; Fox *et al.*, 2008); reaction time, balance, neurotransmission, and long term potentiation (LTP) (Lasley and Gilbert, 1999; Lasley and Gilbert, 2002); and other CNS endpoints. Adverse effects on some of these CNS endpoints have been produced by blood lead concentrations in animals as low as 12 μ g/dL (Fox *et al.*, 2008). While these data provide a fuller picture of the CNS damage that may occur from lead exposure, a summation of the data from these related endpoints was not thought to be necessary to demonstrate that there is sufficient *in vivo* evidence to support the observed epidemiological associations between early life lead exposure and decrements in the IQ of school-aged children.

Therefore, only evidence from *in vivo* neurobehavioural tests of cognitive function is summarized here. For a more complete recent summary of the *in vivo* evidence of the full spectrum of the CNS effects of lead exposure readers are referred to US EPA (2006).

Additionally, it is not possible to summarize all of the experimental animal literature on the neurobehavioural effects of lead here. There is a very large body of the literature on the subject and an exhaustive summary is unnecessary to establish that there is sufficient *in vivo* evidence to support the findings of the epidemiological studies. Instead, what is presented here is a summary of the most relevant, high quality studies of the neurobehavioural effects of environmentally relevant doses. These studies, while only a portion of the literature on this subject, provide sufficient evidence of the neurobehavioural toxicity of lead in laboratory animals. On balance, the remainder of the literature also supports the conclusions of the subset reviewed here, however many of these additional studies were conducted at higher doses or suffer from methodological shortcomings, such as poor control of weight related effects or very small numbers of animals. A more comprehensive summary of the experimental literature was recently completed by the US EPA (2006). An exhaustive summary would not change the conclusions drawn from the more limited review presented here.

- Several general observations emerge from the collective experimental literature on the neurobehavioural effects of lead. These are:
- The neurobehavioural effects of lead appear to be not only dose dependent, but also dependent on the timing of exposure. For some endpoints, perinatal exposures may not be more damaging or potent that equivalent exposure doses later in life.
- Learning in general and performance on difficult behavioural tasks are more sensitive to lead-induced impairment than performance on easier tasks.

- There is a great deal of inter-individual variability on response to the same environmental exposure doses under similar experimental conditions. At lower exposure doses for some endpoints, lead appears to push more individuals into the lower range of functioning that is observed within the control animals. Or, to put it another way, the prevalence of what might be considered extreme, or abnormal responses to behavioural testing among the control animals, is increased within the lead exposed groups. This variable, graded response is consistent with the epidemiological literature.
- It is important to consider the longitudinal nature (pattern of effects over time) of the potential neurobehavioural effects of lead. Impairments in behavioural test performance may only become apparent over the course of many test sessions (e.g. the pace of learning or adapting may vary, or the apparent dose-response relationship may change).

These general observations from the results of *in vivo* experimental studies with laboratory animals help explain some of the inconsistencies of the epidemiological evidence. It is clear from the animal studies that the neurological effects of lead are subtle and complex.

Adverse neurobehavioural effects of lead exposure have been demonstrated in multiple animal species, including:

- Impaired learning and motor coordination in herring gull (*Larus argentatus*) chicks (Burger and Gochfeld, 1997; Burger and Gochfeld, 2005)
- Impaired learning (visual discrimination) in prenatally lead exposed lambs (Carson *et al.*, 1974)
- Altered spatial exploration in offspring of paternally lead exposed rabbits (Nelson *et al.*, 1997)

- Decreased running speed and maze learning in short-tailed opossum (*Monodelphis domestica*) (Punzo and Farmer, 2004)
- Increased aggressive behaviour in golden hamster (*Mesocricetus auratus*) (Delville, 1999) and
- Impaired social development, including increased male aggression, in offspring of maternally exposed mice (Donald *et al.*, 1987)

The most extensive and informative literature, however, is from experiments with non-human primates and rats. These two animals will be the focus of the subsequent review of the *in vivo* evidence of neurobehavioural effects of lead exposure.

Data from non-human primates is more directly relevant to neurodevelopmental effects on humans because rodents develop from infancy to adulthood over a period of weeks, whereas the neurological development period of non-human primates is more protracted and analogous to that of humans.

3.3.1 Experimental Studies with Non-human Primates

There are at least 17 published reports of adverse neurobehavioural effects of lead in experiments with non-human primates. Not every endpoint examined, nor every exposure dose, nor every exposure period was associated with adverse outcomes. However, only one publication that reported primarily negative results was located (Laughlin *et al.*, 1999). The preponderance of evidence supports a causal relationship between lead exposure and neurobehavioural impairments in experiments with non-human primates. Adverse effects, however, are dependent on the difficulty of the task, domain tested, lead exposure period and dose. Adverse effects have repeatedly been reported in non-human primates with a history of lead exposure that produced mean blood lead concentrations in monkeys as low as 11 to 15 μg/dL. Most studies were done using oral exposure to lead acetate in drinking water, gelatine capsules, or milk. No threshold for neurodevelopment effects of lead exposure in non-human primates has been clearly established.

A significant portion of the literature on the behavioural effects of lead on monkeys comes from work conducted at Health Canada laboratories by Dr. Deborah Rice and colleagues. This body of work forms the focus of this review. Additional supporting studies are also summarized where they were viewed as providing important additional evidence.

The primate studies by Dr. Rice summarized below were all done with the cyanomolgus monkey (*Macaca fascicularis*). The results of earlier, relatively high dose studies are not emphasized here. The results of three more recent experimental designs are given more weight. These are: (1) low dose; (2) moderate dose, variable exposure; and (3) high dose, repeat testing. The design and results of these experiments are explained in more detail below

Low dose

The studies conducted by Dr. Rice and colleagues at Health Canada provided the lowest published blood lead concentrations in monkeys associated with adverse behavioural effects. At the time of the experiment, the blood lead concentrations among the monkeys in the low exposure group were not much higher than those in environmentally exposed humans. Monkeys in this study were exposed from birth on, but were not tested until they were three to four years old. Cynomolgus monkeys (*Macaca fascicularis*) were orally exposed five days per week to 0, 50, or 100 μ g/kg/d lead as lead acetate in milk solution or gelatine capsules from birth onwards in mixed sex dose groups of at least five animals. Mean (SE) blood lead concentrations peaked at 3.5 (0.6), 15.4 (1.7), and 25.4 (2.0) μ g/dL, respectively and

declined to steady state concentrations of 2.9 (0.5), 10.9 (0.7), and 13.1 (1.4) µg/dL, respectively. No lead related effects on weight were reported (Rice, 1985). The blood lead concentrations in these monkeys was lower than previously published studies of the behavioural effects of lead on non-human primates. Monkeys from this experiment were, starting as juveniles, tested on a variety of behavioural tasks, including spatial and non-spatial discrimination reversal, delayed spatial alteration, differential reinforcement of low rate (DRL), and fixed interval (FI) and fixed ratio (FR) schedules of reinforcement.

Fixed Interval

Fixed Interval (FI) is a simple reinforcement schedule in which reward follows the first response following a fixed time interval, such as one minute, and then the first response following a subsequent interval, and so on.

The low dose monkeys from the Health Canada laboratory were assessed at three to four years old on an eight minute Fixed Interval (FI) schedule of reinforcement (Rice, 1984b). The lead-exposed monkeys (all dose groups) initially had lower and less variable median inter-response times (a higher rate of un-rewarded responding) than control monkeys. This lead effect on learning was only observed over the first 20 sessions. There was no difference in FI responding by 60 to 80 sessions. Lead-treated monkeys also had less consistent performance during the initial sessions – as demonstrated by greater intra-individual variability of the response rate. There was no reported effect of lead on post-reinforcement pause times.

Differential Reinforcement of Low Rate (DRL)

Following the FI testing above, the monkeys were assessed on a Differential Reinforcement of Low Rate (DRL) schedule (Rice, 1985). DRL requires subjects to

withhold responding for a specified interval to be rewarded. Rice (1985) reported the that monkeys were trained on 5, 10, and 20 second delay schedules and tested on 30 second delay schedules. Linear trend analysis indicated that the performance of lead exposed animals did not improve as rapidly as controls. Lead exposed animals had a lower rate of increase in reinforced responses and had a slower rate of decrease in unreinforced responses. Lead exposed monkeys also had a higher rate of between-session variability over the latter sessions. There were no differences between groups in number of reinforcements, mean or median interresponse times, or post-reinforcement pause times. In a latter experiment from the same laboratory, monkeys exposed to higher lead doses also demonstrated impaired DRL performance when tested as adults (Rice, 1992a).

Nonspatial discrimination reversal

Monkeys were tested on 3 discrimination reversal tasks at 3 years of age: (1) nonspatial form discrimination; (2) nonspatial colour discrimination with irrelevant form cues; and (3) nonspatial form discrimination with irrelevant colour cues (Rice, 1985). A dose-related impairment in performance was reported for most, but not all endpoints. The reported LOAEL for nonspatial colour discrimination with irrelevant form clues and combined errors in the last four reversals of all three tasks was 50 μ g/kg/d – associated with a mean peak blood lead in infancy of 15 μ g/dL and a mean concurrent blood lead of 11 μ g/dL. Lead induced impairment of performance on spatial and non-spatial discrimination tasks among infant monkeys was also reported by Bushnell *et al.* (1979) at significantly higher lead exposure doses. In a subsequent experiment from the same Health Canada laboratory, monkeys with slightly higher blood lead concentrations displayed a greater degree of impairment on these three discrimination reversal tasks (Rice and Gilbert, 1990b). However, in a subsequent experiment where monkeys had even greater blood lead concentrations, the effects were somewhat reversed (Rice, 1992a) and the results were similar to

those reported above. This apparent inconsistency in results was attributed to exposure of the monkeys in the latter study to a reversal task (nonspatial discrimination reversal) during infancy.

Delayed spatial alteration

A delayed alteration task was administered to the same animals at seven to eight years of age to test for deficits in spatial learning and memory (Rice and Karpinski, 1988). Monkeys were required to press a lit lever for reinforcement and advancement. The correct lever was alternated between trials with increasing (up to 15 seconds) temporal delay between trials. The delay is introduced to test spatial memory. The number of incorrect responses and session length were identified as the primary study endpoints. Statistically significant increases in errors per session and increased session length were reported for the low dose group (50 µg/kg/d; concurrent mean blood lead 10.9 µg/dL) at delays of five seconds and longer. The authors also noted severe preservation behaviour among 25% of the low dose group and 80% of the high dose group during trials with the 15 second delay – with some animals persistently responding on the incorrect lever for hours at a time. This behaviour was not observed among the control animals. The authors also note that the degree of impairment on the delayed alteration tasks by the lead exposed monkeys was comparable to that displayed by monkeys that had extensive lesions in the frontal cortex of their brains. In a latter experiment from the same laboratory, monkeys exposed to higher lead doses were unimpaired as adults on a delayed spatial alteration task (Rice, 1992a). This apparent inconsistency in results was attributed to exposure of the monkeys in the latter study to a reversal task (nonspatial discrimination reversal) during infancy.

Spatial discrimination reversal

Monkeys were tested at nine to ten years of age on a series of spatial discrimination tasks with and without irrelevant form and colour cues (Gilbert and Rice, 1987). Lead exposed monkeys were impaired in the spatial discrimination tasks with irrelevant form and colour clues only. Performance of the low exposure group was only impaired on the first task after introduction of the irrelevant cues, but not after they became familiar with the irrelevant cues.

Constant Dose, Variable Exposure Periods

In a second series of experiments from the Health Canada lab, the potential neurobehavioural effects of a single constant lead dose at different periods of development was tested (Rice and Gilbert, 1990b). The lead exposure dose in this experimental design was slightly higher than that used in the low dose experiment above. Monkeys were assigned to one of four exposure groups of 13 animals each: (1) control; (2) continuously lead exposed from birth; (3) lead exposed during infancy only (up to postnatal day (PND) 400); or (4) lead exposed after infancy only (after PND 300). Lead treated animals received an oral dose of 1.5 mg/kg/d. The mean blood lead concentrations of monkeys not exposed to lead, exposed and consuming formula, and exposed after weaning were 3 to 6 µg/dL, 32 to 36 µg/dL, and 19 to 26 μ g/dL, respectively. Monkeys were assessed on a variety of behavioural tasks, including spatial and non-spatial discrimination reversal, delayed spatial alteration, spatial discrimination reversal, concurrent discrimination performance, and FI and FR schedules of reinforcement. The archived brain tissue of 23 year old monkeys exposed during infancy only was also analyzed for biochemical and pathological features that are characteristic of Alzheimer's Disease.

Nonspatial discrimination reversal tasks

Monkeys from this exposure regime were tested at five to six years old on the same nonspatial discrimination reversal tasks described above for Rice (1985) (Rice and Gilbert, 1990b). The infancy only exposure group (3) did not demonstrate any lead related impairments, despite the higher lead exposure relative to the animals in the lower lead exposure study (Rice, 1985). The other two exposure groups (continuous and post infancy) were impaired in all tasks. The overall sensitivity of exposure period was continuous > post infancy > infancy only.

Delayed spatial alteration

Monkeys were tested at six to seven years old on a delayed spatial alteration task (Rice and Gilbert, 1990a). All three lead exposure groups were impaired to a similar degree on task acquisition and indiscriminant responding at the longer delay intervals. Lead related inappropriate responding during delay and persistent responding was also reported.

Spatial discrimination reversal

Monkeys were tested at seven to eight years old on a series of spatial discrimination tasks with and without irrelevant form and colour cues (Rice, 1990). In the absence of irrelevant cues, only the continuous exposure group (2) was impaired. In the presence of irrelevant cues, all three exposure groups were impaired to a similar degree. These results are in agreement with those from the delayed spatial alteration test and in contrast to the results of the nonspatial discrimination tests, where monkeys exposed only in infancy were less susceptible. This suggests that spatial and nonspatial effects may be differentially affected depending on the period of lead exposure.

Concurrent discrimination performance

Monkeys were assessed at eight to nine years old on concurrent discrimination performance. This task required the animals to learn a set of six problems concurrently (Rice, 1992b). All three lead exposure groups demonstrated decreased learning speed. Again, the monkeys exposed in infancy only were less affected than the other two exposure groups. The overall sensitivity of exposure period was continuous > post infancy > infancy only.

Fixed Interval and Fixed Ratio

Monkeys were assessed on FI and fixed ratio (FR) schedules at three years of age and again at seven to eight years of age (Rice, 1992c). FR is a simple reinforcement schedule in which reward is delivered after a fixed number of responses, and is delivered again after the same number of responses, and so on. No significant leadrelated effects on FR were reported for all assessment periods. No significant leadrelated effects on FI were observed at three years of age. These results are in contrast to the increased FI response observed in the earlier, lower dose experiment (Rice, 1984b). At seven or eight years of age, lead exposed monkeys had increased response rates relative to controls. There were no significant differences between exposure periods. The author noted that the negative results of the first assessment and positive results of the second may be the result of an interaction of lead with the specific behavioural history of the animals in this experiment. FR also appears to be less sensitive to lead induced impairments than FI.

Alzheimer's Disease

TOXICOLOGICAL REVIEW AND RECOMMENDED TRVS FOR ENVIRONMENTAL LEAD EXPOSURE IN CANADA

Archived adult brain tissue of the monkeys exposed in infancy only (up to PND 400) was recently analyzed for the presence of neurochemical biomarkers and pathological features characteristic of Alzheimer's disease (AD) (Wu *et al.*, 2008). Blood lead concentrations in treated animals had returned to background (control) by adolescence. Archived tissues from animals terminated at 23 years of age were examined by pathological staining as well as for lead concentration and molecular and biochemical markers characteristic of AD. There was no difference in lead concentration in brain tissue at termination between exposed animals and controls. However, ß-amyloid precursor protein (APP) mRNA, transcription factor Sp1, and APP were all elevated in the terminal brain tissue of primates exposed to lead as infants. These results confirm earlier findings from rat experiments (Basha *et al.*, 2005). The amino acid sequence of cynomolgus monkey APP is homologous (96%) to that in humans and ß-amyloid (Aß) peptides form plaques in both species, whereas rodent Aß is not plaque-forming. Brain tissue pathology of the lead exposed monkeys was also observed to be similar to that of a human brain with AD.

Repeated Testing

In this third experiment, monkeys were exposed to a relatively high dose of lead starting at birth and were repeatedly assessed for neurobehavioural effects at three different life stages (Rice, 1992a). Groups of six mixed sex monkeys were dosed continously from birth with 0 or 2,000 µg/kg/d lead as lead acetate via infant formula or gelatine capsules. Blood lead concentrations of exposed monkeys peaked at 115 µg/dL and declined to steady state blood lead concentrations of 33 µg/dL after withdrawal of formula. Monkeys were tested as infants (starting at PND 60), juveniles (2.5-3 years old) and adults (7-7.5 years old) on various tasks that had been identified as sensitive to the behavioural effects of lead. Infants were tested on nonspatial discrimination reversal. Juveniles were also tested on nonspatial discrimination reversal (with and without irrelevant cues). Adults were tested on

DRL, delayed spatial alteration, and learning visual discrimination tasks. In this experiment, there was no impairment on nonspatial discrimination reversal when the monkeys were tested as infants, whereas impairment in the presence of irrelevant form cues was evident when tested again as juveniles. Adult monkeys were found to be impaired on DRL and in their learning of the visual discrimination task, but not on the delayed spatial alteration task.

Monkeys were tested on FR and FI schedules as infants (starting at 75 days old) and juveniles. As infants, lead-treated monkeys had increased mean pause times with the effect increasing with increasing FR value. As juveniles, lead-exposed monkeys had shorter mean inter-response times during the intermediate sessions and increased between session variability in run rate. As infants, FI pause was lower in lead-treated monkeys in latter test sessions and between session variability in pause time was greater in lead-treated monkeys. As juveniles, lead-treated monkeys had a higher mean run rate and greater between session variability in pause time.

These experiments, despite limited statistical power from low sample size and high variability, demonstrated the adverse effects of lead on sensitive measures of operant conditioning after lead exposure in infancy as short as 75 days (this age corresponds to about a 10-month old human infant). The effect on FI performance was age dependent. Increased variability on FI performance in early, but not late, sessions among lead-exposed monkeys suggests a lead-related impairment of acquisition of performance (learning). The effect on FR was also age dependent, with lead-treated animals displaying an increase in post-reinforcement pause time as infants, but not as juveniles. The authors cite a difference in history of scheduled conditioning as the most likely explanation for this age dependent difference in effect. Lead-exposed animals exhibited increased variability of both within session and between session performance. Further, individual monkeys were differentially impaired on different tests. This reflects an endpoint-dependent variance in

individual susceptibility to the adverse neurological effects of lead. This experiment also provided additional data to support the hypothesis that FI effects are more sensitive to lead than FR effects and that schedule-controlled behaviour may be more sensitive to lead-induced impairment than other types of behavioural tasks.

Earlier Health Canada publications

Dr. Rice published an earlier series of reports with higher lead exposures and fewer animals. Eight monkeys (four control, four treated) were orally exposed continously from birth to 500 ppm lead as lead acetate in infant formula or in gelatine capsules. Blood lead concentrations of exposed monkeys peaked at 50-60 μ g/dL and declined to steady state blood lead concentrations of 25-35 μ g/dL after withdrawal of formula. Rice (1984a) reported impaired function on nonspatial and spatial delayed matching to sample tasks among the lead-exposed monkeys at three years of age. No lead related effects on locomotor activity were reported, but lead exposed monkeys had increased FI 8-min response rates (Rice *et al.*, 1979).

Additional non-human primate studies

Laughlin *et al.* (1999) tested the early neurological development of female rhesus monkeys (*Macaca mulatta*) exposed to lead in infancy. Starting on PND 8, leadexposed monkeys (n = 48) were orally administered 4 ml solution of lead acetate via syringe. Blood lead concentrations of lead-exposed monkeys achieved about 20 µg/dL by week four. Monkeys were tested once per week for four weeks with the Schneider Neonatal Assessment for Primates (SNAP). There were no systematic relationships between blood lead level and performance on the test. Lasky *et al.* (2001) tested the effects of postnatal lead exposure on the spatial exploration behaviour of the same female rhesus monkeys as described above. Starting on PND 8, lead-exposed monkeys (n = 48) were orally administered 4 ml solution of lead acetate via syringe. The lead dose was adjusted to achieve a target blood lead of 35-40 µg/dL. It took 12 weeks for the blood lead concentrations of the exposed monkeys to increase to this target concentration from the initial concentration of 15 µg/dL. Evaluation of spatial exploration behaviour started at two weeks of age and continued up until weaning at 26 weeks. Lead-exposed monkeys exhibited more fear, agitation, and climbed the wire sides of the cages more frequently than controls during the first exploration session. In subsequent sessions, the lead-exposed monkeys explored the periphery of the cage and escaped (climbed out) more frequently than controls – differences for the latter behaviour did not reach statistical significance.

Significant adverse neurobehavioural effects of lead exposure have been reported in a number of additional experiments with non-human primates. The lead doses or the blood lead concentrations of exposed animals were, however, greater than those of the studies summarized above.

3.3.2 Experimental Studies with Laboratory Rats

There are at least 28 published reports of adverse neurobehavioural effects of lead in experiments with laboratory rats. Not every endpoint examined, nor every exposure dose, nor every exposure period was associated with adverse outcomes. However, only 6 publications were located in the literature that reported primarily negative results. The preponderance of evidence supports a causal relationship between lead exposure and neurobehavioural impairments in experiments with laboratory rats. Adverse effects, however, appear to be dependent on the difficulty of the task, domain tested, lead exposure period and dose. Adverse effects have repeatedly been reported in laboratory rats with a history of lead exposure that produced blood lead as low as 15-20 μ g/dL. A single study additionally reports adverse effects associated with blood lead concentrations as low as approximately 10 μ g/dL (Cory-Slechta and Thompson, 1979). Most rat studies have used oral exposure to lead acetate in drinking water. No threshold for neurodevelopment effects of lead in laboratory rats has been clearly established.

Many of the published experiments on the low dose behavioural effects of lead on rats have been done at the University of Rochester laboratories of Dr. Debora Cory-Slechta. This body of work forms the primary focus of this review. Additional supporting studies are also summarized where they were viewed as providing important additional evidence.

Minimum Response Duration

Male weanling Sprague-Dawley rats were exposed to lead acetate in drinking water at 0, 100, or 300 ppm for 55 days prior to training on a differential reinforcement of minimum response duration task (Cory-Slechta *et al.*, 1981). In this task, animals are required to depress a lever for a specific duration to receive a food reward. No blood lead data were reported. Lead exposed rats had shortened response durations, increased post inter-trial interval latencies, and increased variability of response durations. Introduction of a tonal signal failed to improve the performance of leadexposed rats.

Fixed Interval

In a series of experiments, Cory-Slechta *et al.* (1983; 1985) exposed male weanling Long-Evans hooded rats to 25 ppm sodium acetate (controls), 25 ppm lead acetate (n=12), 50 ppm lead acetate, 100 ppm lead acetate, or 500 ppm lead acetate in

drinking water. Blood lead concentrations among the 25 ppm group averaged between 15 and 20 µg/dL over the course of the behavioural testing and the blood lead concentrations among the 50 ppm group averaged between 20 and 30 µg/dL. There were no lead related differences in animal weights. Behavioural training began at PND 50. Performance was assessed on a one minute fixed interval (FI 1) schedule of food reinforcement over 90 sessions. Lead related effects of FI 1 were dose-dependent. Response rates among the lower two exposure groups were initially higher than controls and then declined toward control rates over the course of the 90 sessions. Response rates among the 100 ppm and 500 ppm groups started equivalent to controls. The rates of the 100 ppm group rose and peaked at around session 60, then declined; whereas the response rates of the 500 ppm group continually rose. This dose-dependent pattern of effects is illustrated in Figure 6. The LAOEL for increased FI 1 response rate was 25 ppm lead acetate in drinking water (14.3 ppm lead) – associated with a blood lead of 15-20 µg/dL. Increased response rates were a product of shorter inter-response times and increased running rates.

Cory-Slechta *et al.* (1979) also reported increased FI 30-sec response rate and variability among male weanling Sparague-Dawley rats exposed to 50, 300, and 1,000 ppm lead acetate in drinking water. Behavioural training began at PND 50-55. Again, the pattern of response was dose-dependent. Significant increases in FI response rate were reported over latter sessions for the 50 ppm exposure group – these rats had a mean blood lead of less than 10 μ g/dL (Figure 1, Cory-Slechta *et al.* 1979). Areola *et al.* (2001) also reported an increase in FI-1 response rates among post-weanling rats orally exposed to 50 ppm lead acetate. This effect was reversed with administration of quinpirole, a D₂ receptor agonist.



Figure 6. Reproduced from Cory-Slechta et al. (1985). Changes in Fl response rate over time as a function of lead acetate exposure concentrations. Each data point represents the group median response rate expressed as a percentage of the control median.

Fixed Ratio

Cory-Slechta (1986) exposed male weanling Long-Evans hooded rats to 50 or 500 ppm sodium acetate (controls), 50 ppm lead acetate or 500 ppm lead acetate. Blood lead concentrations among the 50 ppm lead acetate averaged $30.3 \mu g/dL$ over the course of the behavioural testing and the blood lead concentrations among the 500 ppm group averaged between 58 and 94 $\mu g/dL$. There were no lead related differences in animal weights. Behavioural training began at PND 50. The 500 ppm exposure group displayed decreased response rates over the first sessions of the

FR5 and FR25 response schedules. No other lead effects were reported. The authors noted that FR testing seemed less sensitive to lead effects than the FI tests.

Brockel *et al.* (1998) reported increased response rates and shorter waiting times on a FR waiting-for-reward schedule among male weanling Long Evans rats chronically exposed to 150 ppb lead acetate in drinking water with blood lead concentrations of 26.2 μ g/dL. Acute IP administration of the D₂ agonist quinpirole reversed these effects (Brockel and Cory-Slechta, 1999b). No adverse effects were observed among rats exposed to 50 ppb lead acetate (blood lead <5-9.7 μ g/dL). On this schedule, an additional reward was provided for waiting for an interval following successful completion of the FR task.

Repeated acquisition and performance

Cohn *et al.* (1993) conducted a series of repeated acquisition and performance schedules to test spatial learning and memory. Male SD weanling rats were exposed to 0, 50 or 250 ppm lead acetate in drinking water and tested starting at PND 55. Blood lead concentrations in the exposed animals reached 25.1 µg/dL and 73.5 µg/dL, respectively. Animals were trained on a multiple repeated acquisition and performance schedule beginning at 55 days of age. Repeated acquisition task required the animal to learn a novel pattern of responses each day; whereas the repeated performance tasks required the animal to repeatedly perform the same pattern of responses. Lead-induced impairment was observed only on the repeated acquisition task and this impairment was observed to be related to preservation behaviour, and endpoint that has also been shown to be susceptible to lead related effects in monkeys.

Sustained attention

Brockel and Cory-Slechta (1999a) investigated the effects of chronic lead exposure on sustained attention in male weanling Long Evans rats. Animals were exposed to 0, 50 or 150 ppm lead acetate in drinking water. The lead-exposed animals achieved blood lead concentrations of 16.0 and 28.0 μ g/dL, respectively. The sustained attention task required animals to monitor a cue for reward while simultaneously subjected to an irrelevant light cue. Lead exposure did not result in any significant impairment in performance on this task.

Life-Stages

Dr. Cory-Slechta and colleagues conducted a series of experiments to examine the relative sensitivity of lead exposure during different life stages (Cory-Slechta and Pokora, 1991; Cory-Slechta *et al.*, 1991). Weanling (21 day old), adult (8 month old) and older (16 month old) Fischer 344 (F-344) rats were exposed to lead in drinking water for 8.5 months. Three dose groups were tested at each age: weanling rats were exposed to 0, 2, or 10 mg lead acetate/kg/bw and adult and older rats were exposed to 0, 1.9, or 9.3 mg lead acetate/kg/bw. Different doses were used between the young and older rats to try and produce equivalent brain lead concentrations.

Rats were tested on a delayed spatial alteration performance task after four months of exposure (Cory-Slechta *et al.*, 1991). Delayed spatial alteration performance was improved in young and old rats and relatively unaffected in adult rats. The improved performance was interpreted as preservation of alternating behaviour that had been learned during an extensive task training period. Lead induced preservative behaviour has also been demonstrated in monkey studies.

A differential response across life stages was also observed for results of testing on FI and variable interval (VI) schedules (Cory-Slechta and Pokora, 1991). Young and old rats displayed increased response rates in FI and VI tasks, while adult rats had

decreased response rates. FI appeared to be more sensitive to lead-induced impairments. The authors also noted that the F-344 rats appeared to be less sensitive at these lead doses than Long-Evans rats from prior experiments.

Additional Rat Studies

There are dozens of additional in vivo studies that could be reviewed and summarized. However, doing so would not change the overall conclusions supported by the above evidence - that lead exposure in laboratory animals producing blood lead concentrations between 10 and 20 µg/dL causes adverse neurobehavioural and other neurological outcomes. Therefore, the inclusion of additional studies in this summary section was limited to those studies that examined the effects of relatively low lead exposure (defined as blood lead concentrations < 20 μ g/dL). The focus is on presenting evidence of effects from the lower range of experimental exposures because it is clear from the larger body of in vivo evidence that the pattern of lead-induced neurotoxicity is dose dependent. Therefore, results from higher exposure studies are of more lomited relevance. It should be further noted that the selection of evidence presented in this section is not exhaustive; rather it is intended to be representative of the nature of evidence presented in the literature. The evidence presented in this section is drawn primarily from a series of experiments designed and executed in Dr. Barbara Strupp's laboratory at Cornell University to: (1) elucidate the specific cognitive processes that are affected by low-level lead exposure; (2) explore the mechanisms by which lead impairs cognitive function; and, (3) evaluate the efficacy of oral chelation to mitigate the neurotoxicity of lead.

Chronic adult exposure and delayed spatial alteration

Alber et al. (1996) demonstrated impairment on a delayed spatial alteration (DSA) task with variable inter-trial delay among rats chronically exposed to lead with median blood lead concentrations of 19 µg/dL. Mature female Long-Evans rats were exposed to 300 ppm sodium acetate (control), 75 ppm lead acetate, or 300 ppm lead acetate in drinking water starting at three weeks of age. Dose groups consisted of 13-15 animals. The median blood lead concentrations at 20 weeks was <5, 19 and 39 µg/dL, respectively. The animals were 52 weeks old when training on the spatial alteration task began – they had been previously trained and assessed for sustained attention, response inhibition and distractibility. The lead-exposed rats were not impaired in learning the spatial alteration task (no inter-trial delay) but both the 75 ppm and 300 ppm dose groups had reduced performance, relative to controls on the DSA task. The relative impairment of the lead-exposed animals was constant across the delays periods, suggesting that an impairment in short-term memory is likely not responsible for the observed effects. Additional analysis of these study results suggests that the lead-exposed animals were more disturbed by the unpredictability of the inter-trial delay or were more impatient than the control animals. The high exposure animals also exhibited stronger side bias (preference for responding on one side). Deficient response inhibition and preservative behaviour has also been noted in other in vivo experiments.

An interesting finding from the same laboratory is that the above observed leadinduced deficit on the DSA was not observed when rats were exposed to much higher amounts of lead, producing mean blood lead concentrations in the range of 131-157 µg/dL on PND 24, but were only exposed during gestation or gestation and lactation (lead exposure was terminated at PND 30 (Garavan *et al.*, 2000). This suggests that, for this outcome assessed as an adult, relatively low concurrent lead exposure is more potent than relatively high early life exposure. There is evidence of a potential inter-species difference in susceptibility to the neurotoxic effects of lead - at least for overt signs of CNS toxicity. Young rats with blood lead concentrations in the range of 131-157 μ g/dL were asymptomatic (Garavan *et al.*, 2000). Similarly, postnatally lead-exposed rhesus monkeys with blood lead concentrations of 80 μ g/dL who experienced a transient spike in blood lead concentrations to 250-300 μ g/dL at 5-6 weeks old were also asymptomatic (Levin and Bowman, 1986). This is in contrast to human children who are generally considered at risk of encephalopathy at blood lead concentrations of 100 μ g/dL.

Garavan *et al.* (2000) and Morgan *et al.* (2001) also reported that the blood lead concentrations of their rats exposed during gestation and/or lactation only spiked briefly at PND 24 despite constant exposures. The rats were exposed to lead via lactation and drinking water at constant doses. The same effect was observed among rats weaned at PND 21. Subsequent studies demonstrated that the PND 24 peak could be avoided if the lead concentration in the rat's drinking water was reduced during the period PND 21 to PND 30. The authors report that this postnatal spike in blood lead under constant exposure conditions lasts for no more than a week and attributed the spike to increased absorption associated with rapid maturational changes in the gastrointestinal tract during this period of development. Garavan *et al.* (2000) caution that unless investigators measure and report blood lead concentrations during this period (PND 20-21), the animals could experience an unobserved spike in blood lead concentrations. The magnitude of the reported postnatal peak was an approximate three to four-fold increase in blood lead concentration between PND 8-16 and PND 24.

Chronic lifetime exposure and visual discrimination

Morgan *et al.* (2000) reported that lifetime lead exposure producing blood lead concentrations in rats of about 20 µg/dL resulted in impaired learning and reaction

time in a visual discrimination task. The visual discrimination task measures attention to subtle environmental cues, associative ability (learning), and information processing speed. This was a multi-generational study – female F0 generation Long-Evans rats were exposed to 300 ppm sodium acetate (control), 75 ppm lead acetate, or 300 ppm lead acetate in drinking water starting on day 0 of gestation. F1 generation females were exposed to the same drinking water treatment regime as their mothers starting at weaning on PND 21. There were 19-20 animals in each dose group. The median blood lead concentrations in the F1 animals measured at PND1, PND17 and in adulthood were < 5, 22-27.5 and 37.5-51 μ g/dL, respectively. Lead exposure did not affect maternal weight gain, litter size or pup mortality, but both lead exposure groups had lower weight gain among the F1 animals. Behavioural testing began at seven to nine weeks of age. Lead exposure produced a dose-related impairment in learning and proportion of impaired animals (where 1 standard deviation from the mean among the control animals demarked normal from impaired).

Stangle *et al.* (2007) reported deficits in visual discrimination in rats with mean blood lead concentrations of about 13 μ g/dL and showed that these effects can be reversed with oral chelation therapy. This study used a series of visual attention tests to assess sustained attention (visual discrimination with variable delay and cue duration and visual discrimination with random olfactory distraction), inhibitory control, learning or associative ability, and regulation of emotion in rats exposed to lead. Nulliparous Long-Evans rats were exposed to sodium acetate (control) or 300 ppm lead acetate in drinking water from post natal day (PND) 1 to PND 17. On PND 17 the lead in the drinking water of the moderate dose group was reduced to 20 ppm. At PND 30 the pups were weaned and exposure was terminated. Dose groups of 20 female offspring were used in the experiments. Behavioural testing was initiated on PND 62. Mean and standard deviation blood lead in the controls, moderate, and high lead exposure groups at PND 52 were 1.5 (0.1) μ g/dL, 12.6 (0.8)

 μ g/dL, and 31.0 (0.8) μ g/dL, respectively and were all significantly different from each other. The body weight and general health of the moderate lead exposure group did not differ from controls. The moderate lead exposure group learned the visual discrimination task (with and without variable delay) more slowly than controls. Learning was impaired in both the moderate and high lead dose groups, whereas impaired regulation of emotion was significantly impaired only in the high lead dose group.

Stangle *et al.* (2007) also demonstrated that these adverse effects could be reversed by a single 3 week course of oral chelation therapy (from PND 30 to PND 52) with meso-2,3 dimercaptosuccinic acid (DSMA or succimer). The efficacy of chelation therapy in mitigating these effects was dependent on both the magnitude of lead exposure dose and endpoint. The succimer treatment regime used in this study was similar in duration and body weight normalized dose to that used clinically in children, but it was not allometrically scaled to account for potential interspecies differences.

Altmann *et al.* (1993) exposed Wistar rats at different developmental stages to determine the potential differential effects on active avoidance learning (AAL). Rats were fed diets containing 0 or 750 ppm lead acetate. There were four experimental groups: (1) control; (2) perinatal lead exposure: dams were exposed from 50 days prior to mating until PND 16; (3) post weaning exposure; and, (4) continually exposed (perinatal and postweaning). Male offspring were tested starting at PND 70 to 210. The mean blood lead concentrations at testing among the perinatal, postweaning, and continuous exposure groups were 0.26, 16.2 and 14.3 μ g/dL, respectively. The mean brain lead concentrations at testing among the perinatal, postweaning, and continuous exposure groups were <0.01, 0.09 and 0.16 μ g/g wet weight, respectively. On the second of two days of testing, the total number of avoidance reactions was significantly reduced in the perinatal and continuous lead

exposure groups, but not in the post weaning lead exposure group. The authors noted that lead-induced behavioural effects were associated with perinatal lead exposure, even though the brain lead concentrations of these rats were equivalent to controls at the time of testing.

In a series of similar experiments Chen *et al.* (1997; 2001) also investigated the differential effects of lead exposure during different periods of development.

Chen *et al.* (1997) assessed differential effects of exposure period on learning performance. Sprague-Dawley rats were exposed to 0.2% (w/v) lead acetate in drinking water at different developmental stages including a maternally exposed group (perinatal), a postweaning exposed group, and a continuously exposed group. Blood lead concentrations at PND 56 were control: < 2 μ g/dL; perinatal: 3.6 μ g/dL; postweaning: 26.3 μ g/dL; and continuous: 29.6 μ g/dL. Male offspring were tested on two-way active avoidance training. Frequency of avoiding was lower and frequency of not responding was higher in continuously lead exposed rats. No effects were observed among the perinatal and postweaning exposure groups.

Using a similar experimental design, Chen *et al.* (2001), examined the effects of different periods of lead exposure on inhibitory avoidance learning. Male offspring were tested on a step-down avoidance learning task at PND 55-56. In this task,rats were periodically administered a foot shock. Animals could seek refuge on a raised platform. The number of footshocks prior to the animal staying within the refuge area for > 2 minutes was the measurement endpoint. Rats were re-introduced to the test environment without foot shock 24 hours later. The latency to step down was the measurement endpoint for this test of memory. All three lead-exposed groups required a greater number of shocks to reach the criterion than did controls. The order of sensitivity was postweaning> continuous> perinatal. There were no significant differences in latency to step down from the refuge area among the

exposure groups; however, all groups were at or very near the ceiling time limit of the observational period for this outcome.

Subchronic oral lead exposure producing peak blood lead concentrations of 37 μ g/dL produced adverse effects on environmental habituation behaviour of rats (Gong and Evans, 1997). Adult (42 day old) male Fischer 344 rats were exposed to 0, 150 or 2,000 ppm lead acetate in drinking water for 3 weeks. Blood lead concentrations peaked at 37 μ g/dL and 82 μ g/dL, respectively, among the moderate and high exposure groups. The 150 ppm exposure group displayed changes in patterns of environmental habituation (rearing) behaviour when placed in an unfamiliar cage. This effect was reversed by oral chelation with meso-2,3-dimercaptosuccinic acid (DMSA).

Several conclusions are supported by this literature, including

- Blood lead concentrations in rats as low as 12-20 µg/dL can produce adverse neurobehavioural effects and these effects can be mitigated with oral chelation therapy;
- The nature of these effects is dependent on the magnitude and duration of dose, as well as the timing of assessment relative to exposure;
- Rats and monkeys may be less susceptible to the symptomatic neurotoxicity of lead than humans; and,
- Immature Long-Evans rats, with constant lead concentrations in maternal milk and drinking water, demonstrate a 3-4 fold spike in blood lead concentration at PND 20-21.

3.3.2.1 Summary
There is a large body of literature on the *in vivo* neurobahavioural effects of lead exposure in laboratory animals. A representative sub-set of the most relevant studies have been summarized here. The preponderance of evidence supports the conclusion that lead exposure in laboratory animals results in adverse neurobehavioural outcomes. Lead exposure has produced adverse nuerobehavioural effects in at least six different species of laboratory animal and in multiple strains of laboratory rat. At least 17 published studies report adverse neurobehavioural effects in laboratory monkeys exposed to lead. No threshold for these effects has been clearly established and adverse effects have resulted from chronic blood lead concentrations in monkeys as low as10 µg/dL (concurrent) to 15 µg/dL (peak). At least 28 published studies report adverse neurobehavioural effects in laboratory rats exposed to lead. No threshold for these effects has been clearly established and adverse effects have resulted from chronic blood lead concentrations in rats as low as 10-15 µg/dL (concurrent). Neurobehavioural effects in animals have been shown to persist after cessation of lead exposure and blood lead concentrations have returned to normal. Additionally, neurobehavioural effects caused by relatively low level lead exposures in laboratory animals can be mitigated by the administration of oral chelation therapy. It is therefore, concluded that there is sufficient in vivo evidence to corroborate the epidemiological evidence of an association between early life lead exposure and decrements in school-aged IQ.

The collective *in vivo* evidence also illustrates the potential complexity of the lead-CNS exposure-response relationships. Under controlled conditions, these experiments revealed significant inter-individual variability in response. The effect size and direction of response was also dependent on the magnitude, duration, and timing of exposure as well as the life stage at which the outcome was assessed. There is also a tempral pattern to the neurobehavioural effects of lead. These observed complexities under controlled experimental conditions help explain some of the challenges and inconsistencies in detecting a lead-IQ effect in observational studies.

3.4 MECHANISMS OF DEVELOPMENTAL NEUROTOXICITY

The objective of this section is to provide an overview of the evidence of mechanisms of lead's neurotoxicity. The primary reason for reviewing mechanistic evidence in the context of a TRV derivation document is to determine whether the mechanisms behind in *vivo* effects seen in laboratory animals are relevant to humans or if they are specific to the species used in laboratory experiments. In the case of the neurotoxicity of lead, this line of evidence is of diminished importance because a large body of epidemiological evidence of the neurotoxic effects of lead in humans exists – relevance has already been strongly established. An additional limiting factor is the uncertainty of relating *in vitro* exposure-response relationships where the exposure metric is free ionic lead (Pb²⁺) to an equivalent *in vivo* whole blood lead concentration. Nonetheless, a brief overview of the mechanisms of lead neurotoxicity is provided here for completeness and to qualitatively add to the weight of evidence of the *in vivo* and epidemiological lines of inquiry.

Lead has been shown to interact with all cell types in the CNS. There is, to varying degrees, evidence to support a wide range of mechanisms that could result in impaired cellular functioning and survival leading to impaired CNS function. These include lead induced apoptosis; decreased cellular respiration; dysfunction in neurotransmitter synthesis, storage, release, and reuptake; oxidative stress; Ca²⁺ and Zn²⁺ mimicry and associated disruption in homeostatisis and protein function; impaired synaptic plasticity; disturbances in glial cell functioning; dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis and related interaction with the mesocorticolimbic dopamine system; and alteration of the epigenome. These mechanisms and key supporting evidence are discussed in more detail below.

The *in vitro* cellular neurotoxicity of Pb²⁺ has been demonstrated at concentrations in the low picomolar range. Ten μ g lead/dL whole blood is equivalent to 0.48 μ M. However, the equivalent in vivo whole blood lead concentration to a nominal in vitro Pb²⁺ concentration is uncertain. There are several mechanisms that suggest that the free ionic Pb²⁺ available for interaction with cellular targets of the CNS would be less than that measured in whole blood. Most of the lead in whole blood is bound to the erythrocyte and would not be available for interaction with other target cells. At whole blood lead concentrations less than 10 µg/dL, approximately to 99.75% is estimated to be bound to erythrocytes, with the remaining 0.25% is in the plasma and, thereby, more available for distribution to sites of toxicity (Manton et al., 2001). The blood-brain-barrier or the blood-cerebrospinal fluid barrier may also further limit the fraction of lead in whole blood in circulation that reaches cellular targets in the CNS. Manton et al. (1984) report that the lead concentration in the cerebrospinal fluid of lead exposed humans was consistently less than their respective serum lead concentrations and, on average, was about 50% of the serum lead concentrations for those with blood lead < 20 μ g/dL. On the other hand, active transport mechanisms or high affinity intracellular binding sites may increase intracellular lead concentrations relative to that of the systemic circulation or extracellular milieu. As it currently stands, there is considerable uncertainty in relating an *in vitro* Pb²⁺ to an *in* vivo whole blood lead concentration. As a conservative estimate, it may be assumed that the lead concentration within the CNS is 0.125% of the whole blood lead concentration general systemic circulation because of the attenuating effects of the blood-brain-barrier or the blood-cerebrospinal fluid barrier and plasma partitioning. Therefore, a whole blood lead concentration in the general systemic circulation of 10 μ g/dL could be associated with a free Pb²⁺ within the CNS of as low as .006 μ M $(0.48 \ \mu\text{M} \times 0.00125)$ or 6 nM. The *in vitro* cellular effects of Pb²⁺ have been demonstrated at Pb²⁺ concentrations three orders of magnitude lower than this - in the low picomolar range.

Apoptosis

In vitro (He *et al.*, 2000) and *in vivo* (Fox *et al.*, 1997) exposure to lead concentrations in the range of 10 nM to 1 μ M result in apoptosis of rod cells. There are several mechanisms that may lead to apoptosis. Picomolar Pb²⁺ inhibits rod cyclic guanosine monophosphate (cGMP) phosphodiesterase and prohibits cGMP hydrolysis (Srivastava *et al.*, 1995).

Lead also results in increased intracellular Ca²⁺ and Pb²⁺ (Bressler *et al.*, 1999), which are thought to bind to the internal metal binding site of the mitochondrial permeability transition pore, thereby causing mitochondrial depolarization and subsequent activation of cytochrome C-caspase cascade pathway leading to apoptosis. Decreases in retinal Na⁺,K⁺-ATPase activity have also been reported in association with impaired visual function *in vivo* (Fox *et al.*, 1991). In a more recent study, (Fox *et al.*, 2008) showed that lead exposure had a biphasic effect on rod cells of rats exposed *in vivo*: low doses resulted in a proliferative effect and higher doses reduced the number of rod cells.

Oxidative stress

In vitro exposure of O-2A oligodendrocyte precursor cells (OPCs) to1 μ M lead results in suppressed division and increased differentiation. The lead-induced increase in the cellular redox state results in subsequent reduction in density of platelet derived growth factor receptor alpha (PDGFR α) via activation of Fyn and c-Cbl ubiquitin ligase and suppression of Erk1/2 (Li *et al.*, 2007). Suppressed division of the pool of OPCs may lead to reduced myelination and associated CNS effects.

Mimicry of Ca and Zn

Lead's ability to mimic Ca²⁺ can disrupt Ca²⁺ homeostatis and activate Ca²⁺ dependent second messengers. Lead activates the second messenger calmodulin, which in turn stimulates several protein kinases, cyclic AMP and phosphodiesterase, and affects the functioning of Na⁺ channels. At nM concentrations Pb²⁺ substitutes for Ca²⁺ in activating calmodulin but inhibits calmodulin at high concentrations (Kern and Audesirk, 2000). Protein kinase C (PKC) is also activated by Pb²⁺ - PKC mediates many cellular functions, including cellular proliferation and differentiation. Acute *in vitro* lead exposure stimulates PKC at four to five orders of magnitude lower than Ca²⁺ (at picomolar concentrations) but and inhibits PKC at nano and micromolar concentrations (Tomsig and Suszkiw, 1995). It is not clear how these effects translate into chronic effects *in vivo*.

Pb²⁺ may also mimic Zn²⁺. Zn proteins are involved in the regulation of genetic transcription, signal transduction, cell growth and differentiation, and chromosome structure. At concentrations of 2.5 μ M Pb²⁺ interferes with DNA binding properties of Sp1 and TFIIIA by acting at the Zn binding site of these proteins (Zawia *et al.*, 1998; Hanas *et al.*, 1999). The developmental profile of Egr-1 DNA binding has also been altered by *in vitro* lead exposure (Reddy and Zawia, 2000).

Neurotransmission

Lead can interfere with the synthesis, storage, release, turnover, and uptake of neurotransmitters. Lead suppresses the evoked release of acetylcholine, dopamine, and γ -aminobutyric acid (GABA), and causes alterations in glutamatergic synapses. Micromolar Pb²⁺ exposures depress depolarization-dependent neurotransmitter release by inhibiting Ca²⁺ influx via voltage-sensitive Ca²⁺ channels. Low nM concentrations of Pb²⁺, in the absence of Ca²⁺ and depolarization, can stimulate exocytosis of neurotransmitters. The combined outcome of these effects is a

biphasic dose-effect relationship on neurotransmitter release (Lasley and Gilbert, 2002). There are multiple plausible mechanisms by which lead may stimulate exocytosis of neurotransmitters, including activation of CaMKII-dependent phosphorylation of synapsin I, activation of synaptotagmin I, induction of calmodulin-dependent phosphodiesterase (Goldstein, 1993), calcineurin (Kern and Audesirk, 2000), or Na⁺,K⁺-ATPase (Ferguson *et al.*, 2000). Aminolevulinic acid (ALA) inhibits GABA release and possible competing with GABA at receptors. Addiitonal evidence of lead's ability to perturb neurotransmission includes:

- There is *in vitro* and *in vivo* evidence that lead can depress the cholinergic neuronal system and that resulting behavioural effects can be mitigated by administration of nicotine (Zhao *et al.*, 1999).
- McCoy *et al.* (1997) demonstrated the exposure and duration dependent effects of Pb²⁺ on α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor density.
- There is *in vitro* and *in vivo* evidence that lead disrupts the dopamine (DA) system. The effects on DA and D₁ and D₂ receptor density appear to be dependent on exposure dose and timing, sex, brain region, and environmental variables, such as stress (Widzowski *et al.*, 1994; Cory-Slechta *et al.*, 2004).

Synaptic plasticity

Lead may impact long-term potentiation (LTP) by altering glutamate release, postsynapitic *N*-methyl-D-aspartate (NMDA) activation, and neurogenesis.

Chronic *in vivo* lead exposure increases the threshold for LTP induction, decreases LTP magnitude, and accelerates LTP decay in the hippocampal dentate gyrus and the hippocampal CA1, but not the hippocampal CA3 regions (Gutowski *et al.*, 1997; Gilbert and Mack, 1998; Gutowski *et al.*, 1998; Gilbert *et al.*, 1999b; Gilbert *et al.*, 1999a). A biphasic dose-effect relationship has been observed for *in vivo* lead exposure and LTP. Evidence suggests that this may be due to a biphasic effect of lead on stimulated glutamate release (Lasley and Gilbert, 2002).

Hippocampal long-term potentiation is dependent on NMDA receptors. NMDA glutamate receptors are important for synaptic plasticity and learning. Lead exposure alters NMDA receptor density, with the direction of effect possibly dose-dependent. There is evidence that lead inhibits functioning of the NMDA receptor channel complex, however, Lasley and Gilbert (1999) indicate that this is more likely by an indirect mechanism. Drug discrimination techniques suggest that decreased density or sensitivity of NMDA receptors occurs over lower lead exposure ranges (Cory-Slechta, 1995; Cory-Slechta *et al.*, 1996). Finally, chronic lead exposure can modify neurogenesis in the hippocampus of the adult rat (Gilbert *et al.*, 2005; Verina *et al.*, 2007)

Glia

In addition to affecting neurons and neurotransmission, lead has also been shown to affect glial cells, with oligodenroglia apparently more sensitive than astroglia. Glial cells cells provide support and protection for neurons. Lead delays differentiation of glial progenitors (Deng *et al.*, 2001) and can produce hypomyelination and demyelination (Coria *et al.*, 1984). 0.25-1.0 μ M lead acetate reduced glutamate synthetase activity (glial-specific enzyme responsible for uptake of glutamate) (Sierra and Tiffany-Castiglioni, 1991).

The glucose-regulated protein of 78kDa (GRP78) is a chaperone protein responsible for regulation of the accumulation of misfolded proteins via activation of the unfolded protein response (UPR). Dysfunction of chaperone proteins is thought to play a role in the genesis of conformational diseases, such as AD, Parkinson's Disease (PD), prion disease and cataracts, which are all characterized by the aggregation of misfolded proteins. There is *in vitro* evidence that lead binds to GRP78, causes GRP78 aggregation and, thereby, decreases interleukin-6 (IL-6) secretion by astrocytes (Qian *et al.*, 2005; Qian *et al.*, 2007). IL-6 secretion by astrocytes has been implicated in both neurodegenerative and neuroprotective processes.

Hypothalamus and Pituitary

The hypothalamus and pituitary glands are part of the endocrine system and regulate several functions including growth, lactation, kidney function, and reproduction. The hypothalamus and posterior (or neural) pituitary are also neurological tissues. The purpose of this section of the report is to present evidence of the toxicological mechanisms that may contribute to the observed deficits in psychological tests of intelligence (in epidemiological studies) or analogous neurobehavioural effects reported from *in vitro* experiments. Therefore, while the reported effects of lead on growth, sexual development and reproduction, and renal function may have a neurotoxic aetiology (e.g. dysfunction of hypothalamus or pituitary), a review of the evidence of the mechanisms responsible for these reproductive, renal and developmental effects falls outside the scope this section. Dysfunction of the hypothalamus and pituitary glands may have an indirect neurotoxic effect, however, due to the production of potentially neurotoxic stress hormones, or glucocorticoids.

The hypothalamic-pituitary-adrenal (HPA) axis regulates glucocorticoids which, in turn, can influence cognitive function via interaction with the mesocorticolimbic

dopamine system of the brain. Lead has been shown to alter the HPA function as measured by blood corticosterone concentrations in adult rats following maternal (prenatal and lactational) lead exposure. Blood corticosterone concentrations in adult male and female rats were significantly elevated at nine months old, but were significantly reduced in males when measured at 14 month old (females were not tested at this second time point) (Cory-Slechta *et al.*, 2004). Corresponding changes in the mesocorticolimbic dopamine (DA) levels in the nucleus accumbens and prefrontal cortex were also reported. Corticosterone was also significantly lowered in adult male rats exposed post to lead post-weaning (Virgolini *et al.*, 2005).

Epigenetics

Epigenetic effects are those that alter the functioning of the gene, but do not alter the genetic sequence of the DNA. The epigenome is comprised of chromatin and DNA methylation patterns and it plays a role in the regulation of gene expression. The epigenome, therefore, can influence phenotypical variation in toxic response.

Early life exposure to lead in rats and monkeys results in increased &-amyloid precursor protein (APP) mRNA expression, &-amyloid (A&) peptides, and Transcription factor Sp1 in brain tissue late in life, even though brain lead concentrations have returned to normal (control) values (Basha *et al.*, 2005; Wu *et al.*, 2008). These effects were not produced by adult lead exposure in rats, confirming that the upregulation of APP was a latent effect of early life lead exposure. These effects were also accompanied by a decrease in DNA methyltransferase activity, suggesting that they are a result of demthylation of the APP promoter region, increasing the responsiveness of the APP gene to normal physiological processes during aging.

3.4.1.1 Summary of Mechanisms of CNS Toxicity of lead

Lead has been shown to interact with all cell types in the CNS. There is, to varying degrees, evidence to support a wide range of mechanisms that could result in impaired cellular functioning and survival leading to impaired CNS function. These include lead induced apoptosis; decreased cellular respiration; dysfunction in neurotransmitter synthesis, storage, release, and reuptake; oxidative stress; Ca²⁺ and Zn²⁺ mimicry and associated disruption in homeostatisis and protein function; impaired synaptic plasticity; disturbances in glial cell functioning; dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis and related interaction with the mesocorticolimbic dopamine system; and alteration of the epigenome. Cellular effects have been demonstrated *in vitro* at Pb²⁺ exposure concentrations as low as the picomolar range. While the equivalent in vivo whole blood lead concentration is uncertain, it has been conservatively estimated that 10 picomolar Pb²⁺ in cerebrospinal fluid is equivalent to 0.16 µg/dL whole blood lead in systemic circulation. Several cellular and biochemical effects of lead exposure appear to have complex exposure-response relationships that may be biphasic or dependent on duration, magnitude, timing of exposure (for *in vivo* experiments), sex, brain region, or co-exposure to other stressors. In summary, there are several well supported mechanistic explanations for the neurotoxicity of lead this line of evidence corroborates the conclusions of epidemiological studies and experiments examining whole organism effects in lead-exposed laboratory animals.

3.5 VARIABILITY IN SUSCEPTIBILITY TO THE NEURODEVELOPMENTAL TOXICITY OF LEAD

The objective of the following section is to review the evidence of potential variations in susceptibility to the effects of early life lead exposure on IQ.

While research has identified environmental and genetic variables, including nutritional status, ethnicity, and a single nucleotide polymorphism in the aminoluvulinic acid dehydrogenase (ALAD) gene, that may influence the relationship between lead exposure and resulting blood lead concentrations (toxicokinetics), this section is concerned with variation in toxicodynamics - that is, for a given chronic blood lead in children, what is the variance in toxic effects? Several variables may modify the blood lead-IQ biomarker-response relationship. These include genetics, overall health status, and environmental variables, such as co-exposure to other chemicals or stressors and nutritional status. There is some evidence on the modifying effects of genetics and environmental stress or conditions and this is reviewed below.

Genetic Polymorphisms

At the time of this report, no published studies that examined the potential modifying effect of single nucleotide polymorphisms (SNPs) on the blood lead-IQ relationship were located in the literature. There is, however, evidence from an epidemiological study of lead and cognitive function in older male adults that suggests that the neurotoxic effects of lead may be modified by genetic polymorphisms. The inverse association tibia lead concentrations and rate annual rates of decline in Mini-mental State Exam (MMSE) score was significantly worse among carriers of variant alleles of the HFE⁷ gene than among wildtype subjects of the Veteran Affairs Normative Aging Study (NAS) (Wang *et al.*, 2007).

Environmental Stress

⁷ The HFE gene codes for the hemochromoatosis protein

Lead exposure and environmental stress or lower socioeconomic status (SES) have been associated with adverse health outcomes, including neurological effects. Both lead and stress related glucocorticoids act on the mesocorticolimbic systems of the brain. It is, therefore, reasonable to suspect that lead exposure and environmental stress may have interactive effects. Several researchers have explored this hypothesis in both epidemiological and laboratory studies.

In epidemiological studies of early life lead exposure and neurological development a stronger inverse association has been reported sometimes, but not always, for subjects with lower SES. Animal studies have also examined how the environment may modify the neurotoxic effects of lead. Weanling rats housed in an impoverished environment had greater lead-induced impairments in spatial learning than rats with similar blood and brain lead concentrations, but housed in an enriched environment (Schneider *et al.*, 2001). The lead-exposed rats housed in an impoverished environment also had significantly decreased neurotrophic factor gene expression in the hippocampus, and effect absent in the lead exposed rats housed in an enriched environment.

Animal research from Dr. Deborah Cory-Slechta's laboratory has demonstrated that maternal stress can modify the effects of lead in both pregnant dams as well as their offspring exposed in utero. This research suggests that lead exposure can modify the biological effects of stress and that the combined effects of lead and stress are quantitatively and qualitatively different from effects arising from exposure to each in isolation. Dr. Cory-Slechta and colleagues have studied the interactive effects of lead and stress, corticosteroid levels) as well as behavioural outcomes. These studies provide strong evidence for interactive effects between lead and stress, but the pattern of effects is complicated and differs in outcome measured, brain region affected, timepoint of assessment and sex. For example, stress mitigated the effect of lead-induced

reduced FI response rates in male rats; whereas in females lead mitigated the effect of stress-induced increased FI response rates (Cory-Slechta *et al.*, 2004).

Conclusions Regarding Variance in Response

There are data that suggest that several variables may modify the blood Lead concentration-IQ relationship. These variables include genetics, overall health status, and environmental variables, such as co-exposure to other chemicals or stressors and nutritional status. The potential modifying effects of these variables should be acknowledged in the derivation of a TRV for this endpoint and risk assessments of lead exposure.

3.6 BIOMARKERS AND NEURODEVELOPMENTAL ENDPOINTS RECOMMENDED FOR TRVS.

As reviewed in Section 2, there is at least moderately strong evidence that blood lead concentrations less than 10 μ g/dL have adverse effects on the central nervous system, the immune system, the reproductive system, the cardiovascular system, haematopoiesis, and renal functioning. No threshold has been identified for any of these effects and one cannot identify a critical effect, as traditionally defined. Therefore, the limiting factor for TRV development for lead is the certainty with which a blood lead concentration-response relationship can be quantified.

Within the domain of developmental neurotoxicity there are a number of biomarkerresponse relationships that have been quantified in the literature and could potentially form the basis for TRV development. These include maternal blood lead and child IQ; maternal bone lead and child IQ; postnatal blood lead and child IQ; postnatal bone lead and child IQ; postnatal blood lead and child behaviour; and postnatal blood lead and academic achievement. The endpoint for which the blood lead concentration-response has been most often investigated and which can be characterized with the greatest certainty is the relationship between post natal blood lead concentration and IQ decrements in school-aged children.

The relationship between maternal blood lead and IQ decrements in her offspring has not been as well studied and the blood lead concentration-response relationship cannot be characterized with sufficient certainty to derive a meaningful TRV (i.e. the uncertainty around the TRV would be relatively large). Among the six longitudinal studies reviewed here that examined the relationship between maternal blood lead or cord lead and IQ, only one reported a significant inverse relationship (Wasserman et al., 2000a). There are differences among the studies in the time point of collection of maternal blood lead and the timing and instrument used to test IQ and these differences make study to study comparisons difficult; however the existing evidence from the longitudinal data is weighted in the negative direction. The results from analysis of the 696 subjects in the international pooled analysis with cord blood data were also negative for this biomarker-endpoint combination. Maternal and fetal blood lead concentrations, however, are well correlated. And we have no reason to believe that the fetus is less sensitive to the developmental neurotoxic effects of lead than a school-aged child. Therefore, it is recommended that the TRV derived on the basis of the relationship between postnatal blood lead and school-aged IQ should also apply to protection of the fetus and, by extension, to women of childbearing age.

From the current understanding of the toxicokinetics of lead, bone lead is expected to provide a better measure of cumulative lead exposure than blood lead and, therefore, that bone lead would have a stronger relationship to chronic health endpoints than blood lead measurements. The single publication available where this comparison is made supports this position (Wasserman *et al.*, 2003). However, at this point there is insufficient publications on the quantitative biomarker-response relationship for bone lead-IQ relationship (both maternal bone lead and child bone

lead) to derive a TRV for this biomarker-endpoint combination. Recent improvements in the precision of *in vivo* bone lead measurements (Chettle, 2005) may aid in the maturing of this science to a point where derivation of a TRV for bone lead and children's IQ may be possible.

Two issues preclude the development of a TRV for dentin lead and children's IQ. The first are the inconsistencies in both the results and methods of the existing literature. The second is that the environmental lead exposures of the existing dentin lead-IQ studies are all significantly higher than contemporary environmental lead exposures – this raises questions about the validity of extrapolating the reported biomarker-response relationships to lower levels of exposure.

Although the one study to investigate the relationship did report a significant inverse relationship between maternal patella lead and children's neurological development at two years (Gomaa *et al.*, 2002), the endpoint was not IQ (it was BSID MDI) and insufficient evidence exists to support the development of a quantitative biomarker-response relationship for maternal bone lead and children's IQ. Additional studies are required to confirm these results with IQ as an endpoint and to increase the confidence with which a maternal bone lead-children's IQ biomarker-response can quantified.

In summary, the biomarker-endpoint relationship that can be characterized with the greatest certainty for the developmental neurotoxicity of lead is the relationship between postnatal blood lead concentrations and school-aged deficits in IQ. While bone lead shows promise as a complimentary biomarker for the effects of cumulative lead exposure, the bone lead concentration–response relationships have not nearly as well characterized as the blood lead concentration response relationships and are not sufficiently certain to derive a meaningful TRV. The relationship between maternal blood lead concentrations and neurodevelopmental

effects in her offspring is also not as certain as that for postnatal blood lead and IQ. A TRV based on maternal blood lead and IQ in the offspring would also be too uncertain to have any practical value. However, there is no reason to believe that the fetus is any less sensitive to the neurotoxic effects of lead as a school-aged child and maternal blood lead and fetal blood lead concentrations are well correlated. Therefore, the TRV based on the effect of postnatal blood lead concentrations on school-aged IQ should also apply to protection of the fetus and, by extension, to women of childbearing age.

TRVs based on childhood blood lead concentrations and IQ decrements are presented in Sections 5 and 6 of this report.

3.7 SUMMARY AND CONCLUSIONS

This section of the report:

Reviewed the relevant evidence from epidemiological studies on the association between biomarkers of early life chronic lead exposure and developmental neurotoxicity, with an explicit emphasis on the association between children's blood lead and deficits in IQ. The epidemiological evidence is strongly suggestive, though not entirely consistent, of an association between early life chronic lead exposure (as measured by various biomarkers) and decrements in school-aged children's IQ. There is evidence that the developmental neurotoxic effects associated with childhood blood lead concentrations persist out to at least the late teen-age years. Several studies provide evidence of a lack of threshold down to the lowest blood lead concentrations measured in their studies – in the range of 1-2 μ g/dL. There is also evidence of and plausible biological explanations for a steeper biomarker-response relationship at relatively low blood lead concentrations.

Reviewed the available evidence from in vivo experiments on the association between chronic early life lead exposure in laboratory animals and

developmental neurotoxicity. Chronic oral exposure to lead in laboratory animals impairs performance on behavioural tests of cognitive function. These effects have been demonstrated in multiple species including at least two species of non-human primate. Statistically significant impairment on behavioural tests of cognitive function have been reported in animals with average concurrent blood lead concentrations as low as approximately 10 μ g/dL. Neurobehavioural effects in animals have been shown to persist after cessation of lead exposure and blood lead concentrations have returned to normal. Recent findings also demonstrate that some of the lead-induced neurobehavioural effects may be mitigated by oral chelation therapy. No threshold for lead induced behavioural deficits in laboratory animals has been established.

Reviewed the available evidence on plausible mechanisms of action by which chronic early life lead exposure could result in developmental neurotoxicity.

Lead has been shown to interact with all cell types in the central nervous system. Several mechanisms have been demonstrated both *in vitro* and *in vivo* whereby lead exposure that could result in neurotoxic effects. Cellular effects have been demonstrated *in vitro* at Pb²⁺ exposure concentrations as low as the picomolar range. The equivalent *in vivo* whole blood lead concentrations are uncertain, but are likely within the range of those produced by contemporary environmental lead exposures in Canada and possibly 100 times lower.

Reviewed the available evidence on variability in response. There is evidence that environmental stress and genetic polymorphisms may modify the relationship between lead exposure and neurotoxicity.

Justified, on the basis of the most certain biomarker-response relationship, the identification of candidate biomarker-endpoint combinations for TRV development. The endpoint for which the blood lead concentration-response has been most often investigated and which can be characterized with the greatest certainty is the relationship between post natal blood lead concentration and IQ decrements in school-aged children. The biomarker-response relationships for other biomarkers, such as maternal lead, bone lead, and dentine lead, and other neurodevelopmental endpoints, such as behaviour or academic achievement, are relatively uncertain. Therefore, it is recommended that TRVs not be derived explicitly for these alternate biomarker-endpoint relationships. However, there is no reason to believe that the fetus is any less sensitive to the neurotoxic effects of lead as a school-aged child and maternal blood lead and fetal blood lead concentrations are well correlated. Therefore, the TRV based on the effect of postnatal blood lead concentrations on school-aged IQ should also apply to protection of the fetus and, by extension, to women of childbearing age.

SECTION 4 • VASCULAR TOXICITY

4.1 INTRODUCTION

There are multiple lines of evidence, including human epidemiological studies, *in vivo* animal assays, and *in vitro* experiments, that support the hypothesis that chronic lead exposure can result in increased blood pressure in humans. This report does not provide a comprehensive review and analysis of all of the data on this subject because recent, comprehensive summaries of the evidence have been presented elsewhere (IARC, 2006; US EPA, 2006; Navas-Acien *et al.*, 2007; US ATSDR, 2007; Navas-Acien *et al.*, 2008; Vaziri, 2008). All of these reviews support the conclusion that there is sufficient evidence to support a causal relationship between lead exposure and elevated blood pressure in humans.

Lead exposure is associated with adverse effects on variety cardiovascular endpoints including blood pressure, hypertension, inotropic and chronotropic cardiotoxicity, peripheral arterial disease (Navas-Acien *et al.*, 2004), and coronary and cerebrovascular morbidity and mortality (Lustberg and Silbergeld, 2002; Menke *et al.*, 2006; Schober *et al.*, 2006; Navas-Acien *et al.*, 2007; Navas-Acien *et al.*, 2008). However, the endpoint that has been most studied and for which there is the greatest weight of evidence of a causal relationship is lead-induced increases in blood pressure, particularly systolic blood pressure (SBP). Secondly, the other cardiovascular effects may be secondary effects of elevated blood pressure, although there is also evidence of independent and directly cardiotoxic effects of lead. For these reasons, SBP was selected as the critical cardiovascular endpoint for lead. Risk of hypertension and coronary heart disease (CHD) mortality were modeled as a function of SBP to help define an appropriate Benchmark Response (BMR) increase in SBP to derive a toxicological reference value for lead (see Appendix A). There is some evidence of vascular toxicity of lead in children. However, as a whole, this endpoint is considerably less studied in children and the weight of evidence and the quantification of a dose-response relationship are not mature enough to support the derivation of a TRV explicitly on the basis of lead's toxic effects to the vascular system in children. Effects in children will be discussed in more detail in the section below on the variability in susceptibility to the vascular effects of lead. The remainder of the discussion on the vascular effects of lead will be focused on the effects in adult (developed) organisms.

This section of the report will:

Review the available evidence from epidemiological studies on the association between chronic lead and increased blood pressure. The epidemiological evidence of an association between blood lead and elevated BP in adults is inconsistent, but suggestive. Several meta-analyses report a weak, significantly positive association and three of six longitudinal studies published since 1980 report a significant association between blood lead and SBP.

The epidemiological evidence of an association tibia lead and elevated BP is also inconsistent, but suggestive. A significant positive association, after adjusting for covariates, has been found between tibia lead and SBP in three of the seven cohorts where this relationship has been studied.

The epidemiological evidence of an association between lead exposure and elevated BP in children is mostly negative and insufficient to derive a toxicological reference value for this endpoint and lifestage. One good quality study reports an association between lead exposure in children and adverse effects on cardiovascular functioning that could lead to latter life development of hypertension and other cardiovascular morbidity and mortality.

Review the available evidence from in vivo experiments on the association between chronic lead exposure in laboratory animals and increased blood pressure. Both chronic and subchronic oral exposure to lead in laboratory animals results in elevated blood pressure. This effect has been demonstrated in multiple species. The result of more recent animal studies have consistently shown an effect at relatively low levels of lead exposure – statistically significant increases in the blood pressure of rats have been reported at blood lead concentrations as low as 2.4 µg/dL.

Review the available evidence on plausible mechanisms of action by which chronic lead exposure could result in increased blood pressure. The collective evidence from epidemiological studies, *in vivo* and *in vitro* experimental studies clearly indicate that there are several mechanisms by which chronic lead exposure could cause elevated blood pressure and related cardiovascular disease. The mode of action for which there is the most evidence is vasoconstriction secondary to leadinduced oxidative stress and subsequent inactivation of the vasodilator nitric oxide (NO) and related signalling pathways and functional responses.

Review the available evidence on variability in response. There are several variables that appear to be able to confer susceptibility to the effects of lead on SBP and potentially modify the blood lead and tibia lead biomarker-response relationships for this endpoint. These variables include stress, variation in the ATP1A2 gene, and calcium deficiency. These variables may increase the slope of the relationship between blood lead and SBP by up to a factor of 10.

Justify, on the basis of the most certain biomarker-response relationship, the identification of candidate biomarker-endpoint combinations for TRV development. The endpoint for which the blood lead concentration-response has been most often investigated and which can be characterized with the greatest certainty is the relationship between adult blood lead concentration and systolic blood pressure (SBP). The biomarker-response relationships for other biomarkers, such as bone lead, maternal lead, children's blood lead and other cardiovascular endpoints, such as diastolic blood pressure (DBP) and risk of hypertension, are relatively more uncertain. Therefore TRVs were not derived explicitly for these alternate biomarker-endpoint relationships.

4.2 EVIDENCE OF VASCULAR TOXICITY OF LEAD FROM EPIDEMIOLOGICAL STUDIES

There have been a large number of epidemiological studies of an association between human lead exposure and blood pressure or risk of hypertension and many, but not all, epidemiological studies report a significant, but weak association. A significant positive association has been reported among diverse study populations with varied ethnicity and socioeconomic status. A significant positive association remained in many studies after adjusting for variety of potential confounding variables. The modest effect size and the inconsistency of results can be attributed, in part, to measurement error in both lead exposure and blood pressure. The effect of random measurement error in these parameters is null biasing. In contrast to studies that have relied on blood lead as a measure of exposure, bone lead has more consistently been associated with increased blood pressure or risk of hypertension (Navas-Acien *et al.*, 2008). The single study that measured BP with a 24 hour ambulatory monitoring device reported no association with blood lead (Staessen *et al.*, 1996). Another source of variability in outcome is the degree to which the many potentially confounding variables, such as age, body mass index, socioeconomic status, sodium intake, drinking and smoking have been measured and controlled for. Effect modifiers, such as ethnicity or menopausal status, may also add to the noise. On balance, the reported nil associations between human lead exposure and blood pressure or risk of hypertension can equally be interpreted as evidence of the limitations of existing epidemiological investigations to detect an association as they can be interpreted as evidence of an absence of an association.

The following sections present a review of the epidemiological evidence for:

- 1. Blood lead and SBP in adults
- 2. Tibia lead and SBP in adults
- 3. Blood lead and BP in children

Systolic blood pressure (SBP) was selected as the critical endpoint for adults because: (1) SBP has more frequently been associated with lead than diastolic blood pressure (DBP); (2) where they have been reported to have an effect, lead has a stronger effect on SBP; and, (3) SBP may be a more important risk factor for cardiovascular morbidity and mortality than DBP. It is noted that DBP is harder to measure than SBP; therefore, it may only *appear* that lead has a stronger relationship with SBP.

Tibia lead was selected as the bone lead biomarker for adults because results have been reported more often for tibia lead than lead in trabecular bone compartments (patella lead or calcaneus lead) and where they have been both reported tibia lead appears to have a slightly stronger relationship with the critical endpoint (SBP) than does patella lead.

At the time of this report no published studies on the relationship between bone lead and BP or hypertension in children had been located. Therefore, the scope of the review of epidemiological evidence of lead exposure and effects on BP or hypertension in children was limited to studies with blood lead as a biomarker.

It is emphasized that, in consideration of the many limitations of epidemiological investigations, these results need to be interpreted within a weight-of-evidence framework that draws on data from all lines of investigation, including that from animal assays and *in vitro* experiments. A review of evidence from the latter 2 lines of evidence is presented following the summary of epidemiological evidence.

Another point deserving mention in the introduction is that the epidemiological evidence has been characterized as supporting a significant but *weak* positive association between blood or bone lead and SBP (Staessen *et al.*, 1994a; Schwartz, 1995; Navas-Acien *et al.*, 2008). However, even a weak relationship between lead and BP has significant public health implications as both lead exposure and the outcome are very common. So while a slight change in BP may be meaningless for an individual, from a population health perspective it could dramatically change the absolute risk of cardiovascular disease among the population. This is illustrated by the population risk modeling conducted by Health Canada and presented in Appendix A of this document. The results of this modeling indicate that 1 % increase in the mean SBP of Canadians would result in the following population health outcomes:

- The total sex and age-adjusted added risk of hypertension (Pre, Stage I and Stage II) among 35 to 74 year-olds associated with a 1% increase in population mean SBP is approximately 1 in 20 (5,421 per 100,000).
- The sex and age-adjusted cumulative (3 to 4 years) incremental risk of coronary heart disease mortality associated with a 1% increase in population mean SBP is 1 in 2,000 (50 per 100,000).

Blood Lead and SBP in Adults

There is a large body of epidemiological literature examining the association between blood lead and various hypertensive endpoints (SBP, DBP and hypertension). The collective evidence from this literature is inconsistent: Many, but not all epidemiological studies report a significant positive association between blood lead and SBP after adjusting for known relevant confounding variables.

This extensive literature has recently been systematically reviewed by the US EPA (2006) and the US ATSDR (2007). Even though these extensive reviews do not summarize all of the literature on this subject, they are excellent references for readers who require a more detailed survey of the literature than is presented here. The scope of this review is limited to a discussion of recent meta-analyses and longitudinal studies, as meta-analysis and longitudinal studies carry more weight in the interpretation of the evidence. Additionally the recent meta-analyses and longitudinal studies on this subject were judged to be sufficiently representative of the literature as a whole and that a more exhaustive summary of the literature would do little to add to the overall conclusions supported by this more limited review.

Systematic Reviews and Meta-analysis

Nawrot *et al.* (2002) carried out a systematic review and meta-analysis of 31 studies examining the association between blood lead and blood pressure in over 58,000 adult subjects published before February 2001.. Nineteen of the 31 studies were from the general population, four were white collar workers and eight were blue collar workers. The conclusion of their meta-analysis was that there is a weak but statistically significant positive association between blood lead and blood pressure. Of the 48 subpopulations examined by Nawrot *et al.*, 2002 (single study cohorts

were broken out by sex and ethnicity where possible), only 12 reported a statistically significant positive adjusted association between blood lead and SBP (see Figure 7). The meta-analysis, however, did report overall a statistically significant positive association. A statistically significant positive association was also reported for the relationship between blood lead and diastolic blood pressure (DBP), but the effect size was smaller than that of SBP. No significant relationship between the effect size and the mean blood lead concentration of the respective study cohort was reported. The results of the meta-analysis for men and women were not significantly different. However, among the four studies that reported separate results for Caucasians and non- Caucasians, the latter demonstrated a stronger association. African-Americans have higher bone density than Caucasians and, therefore, may have higher body burdens of bone-associated lead.

The results of the systematic review and meta-analysis by Nawrot *et al.* (2002) agreed with earlier systematic reviews and meta-analysis by Schwartz (1995) and Staessen *et al.* (1994a). The Schwartz (1995) analysis reported a 25% higher effect size, but the analysis was limited to data from males only.

Longitudinal Studies

Longitudinal studies are often afforded more weight than cross-sectional studies because they can resolve the issue of temporality (cause precedes effect). A limitation of cross-sectional studies of blood lead and BP is that both of these measures have been in, to some degree, in independent general decline over the past decades. Parallel declines in BP and blood lead could contribute to an observed association among these two measures.

In the case of persistent effects and a relatively transient biomarker of exposure, longitudinal studies are less susceptible to exposure misclassification than crosssectional studies. In the case of lead and BP, these considerations are of less importance because: (1) reverse causation (high BP leads to high lead exposure) seems implausible; and (2) the effects of lead on BP, at least by one mechanism, may not to be persistent. Results from rat studies demonstrate that the hypertensive effects of subchronic and chronic NO-mediated hypertension are not persistent and can be at least partially reversed after up to 3 months of lead exposure with therapeutic treatment with antioxidants (Khalil-Manesh *et al.*, 1994; Malvezzi *et al.*, 2001; Attri *et al.*, 2003). If hypertensive effects of lead via other mechanisms are persistent, they appear not to be induced at these relatively low exposure levels or they require a longer duration of exposure to induce persistent toxicity. These considerations notwithstanding, the available longitudinal studies of lead and BP are reviewed in detail and their respective results are afforded greater weight than the comparatively large number of cross-sectional studies.

Six longitudinal studies of the association between blood lead and SBP published since 1980 were identified (Weiss *et al.*, 1986; Neri *et al.*, 1988; Moller and Kristensen, 1992; Staessen *et al.*, 1996; Glenn *et al.*, 2003; Glenn *et al.*, 2006). Each of these is discussed in more detail below, along with a longitudinal study that included hypertension but not SBP as a study endpoint (Cheng *et al.*, 2001). Three of six longitudinal studies that included SBP as an endpoint reported a significant positive association with blood lead after adjusting for covariates. Two of the three studies reporting a significant positive result were from currently occupationally exposed cohorts; the third positive study was from a formerly occupationally exposed cohort, although the blood and bone lead concentrations in this cohort were comparable to environmentally exposed subjects at the time of the study. The collective evidence from these studies is inconsistent, but supportive of an association between blood lead and increased SBP in adults.

PheeCad, Belgium

Staessen et al. (1996) conducted a longitudinal study on the relationship between blood lead and BP and hypertension among 728 mixed sex (49% men), randomly selected adults (age 20-82 years) in Belgium. The subjects were participants in the Public Health and Environmental Exposure to Cadmium (PheeCad) study, which was a longitudinal follow-up to the Cadmium in Belgium (CadmiBel) study. BP was measured at baseline and follow-up by conventional sphygmomanometry and also at follow-up only by 24 ambulatory monitor. The median follow-up period for this study was 5.2 years; during this period the mean blood lead of the study cohort declined from 8.7 µg/dL to 2.9 µg/dL. Relationships between blood lead and BP or hypertension were explored via multivariate analysis and controlling for age, sex, body mass index, smoking, drinking, physical activity, occupational exposure, social class, menopausal status, use of medications, haematocrit, serum calcium, and 24 hr urinary sodium and potassium. No consistent associations were reported between change in conventional BP and change in blood lead. Blood lead at baseline did not predict onset of hypertension. Nor did the ambulatory BP show a significant relationship with blood lead at baseline or follow-up.

The PheeCad study is, to-date, the highest quality published epidemiological study of blood lead and BP. Great care was taken in the collection of BP measurements. A total of 15 conventional measurements were taken (5 each on 2 separate occasions at baseline and 5 at follow-up). Measurements were taken in the home environment by specially trained nurses with equipment and procedures designed to minimize bias and measurement error. It is the only published study of the association between lead exposure and BP that used 24 hr ambulatory monitoring to measure BP. This method has several advantages over conventional BP sphygmomanometry. The study had one of the largest sample sizes and also had a relatively long period of follow-up. The authors report that the study should have had sufficient power to detect a R^2 as low as 0.04 between blood lead and BP with an α =0.01 and ß=0.90. The study also assessed a wide range of potential confounding variables, including haematocrit, and used biomarkers to assess consumption of alcohol, calcium, sodium and potassium.

South Korean Lead Workers

Lee *et al.* (2001) and Glenn *et al.* (2006) have reported on the relationship between lead biomarkers and BP and hypertension among an occupational cohort employed at 26 lead-using facilities in South Korea. The cohort is 80% male with an average age at baseline (1997) of 40.5 (SD: 10.1) years. The average duration of employment in lead exposed occupations was 8.5 years. Triplicate seated blood pressure measurements were made using a mercury sphygmomanometer at 5 minute intervals. Data was also collected on the following covariates: age, education, hypertension status, body mass index, smoking status, alcohol status, and job duration.

Lee *et al.* (2001) published a cross-sectional study of 798 of the South Korean lead workers and a referent cohort with no occupational lead exposure. Linear regression analysis was used to explore the cross-sectional relationship between lead dose and BP and hypertension. Dimercaptosuccinic acid (DMSA)-chelatable lead was significantly positively associated with SBP. A non-significant (P>0.05) positive association was also reported blood lead and SBP. None of the measures of lead exposure were significantly related to DBP.

More recently, Glenn *et al.* (2006) conducted a longitudinal analysis of this cohort to try and determine whether the effect of lead on BP is acute or chronic. Multiple measures of blood lead, tibia lead and BP were obtained from 575 subjects over 3 years. Longitudinal data were analyzed in multivariate models using generalized estimating equations (GEE). Four models were constructed to explore possible

relationships between acute and chronic lead exposure (represented by blood lead and tibia lead, respectfully) and change in SBP. A significant positive longitudinal association was reported, after adjusting for covariates, between blood lead and SBP. The magnitude of effect (β (95%CI)) of annual increase in SBP was approximately 0.1 (0.01-0.19) mmHg per µg/dL for both cross-sectional blood lead and longitudinal blood lead.

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Figure 7. Reproduced from Nawrot et al. (2001). Change in systolic blood pressure (mmHg) and 95% confidence intervals associated with a doubling of blood lead. Circles represent study subpopulations and squares the combined cohort data. Open circles denote studies where a non-significant association was assumed to be zero. C: Caerphilly Study; HP: Welsh Heart Programe; W: Whites; B: Blacks; NI: non-immigrants; I: immigrants; FW: foundry workers; CS: civil servants; P: Public Health and Environmental Exposures to Cadmium Study.

Normative Aging Study, Boston, USA

Cheng et al. (2001) reported on a prospective study of the association between blood lead and bone lead and the risk of incidence of hypertension among 474 healthy at enrolment, middle aged to elderly, male, predominantly Caucasian subjects in the Normative Aging Study. The mean age of normotensive subjects at baseline was 65.49 (SD: 7.17) years. Mean baseline blood lead was 5.87 µg/dL (SD, 4.01). Blood pressure was measured as the mean of conventional seated sphygmomanometer readings from each arm. Subjects were followed for 1,418 person years. Possible confounding variables included age, age squared, body mass index, family history of hypertension, ethnicity, education, smoking, alcohol use, and dietary intake of Na and Ca. In this study, hypertension was defined as a mean SBP> 160 mmHg, DBP > 95 mmHg, or taking daily medication for the treatment of hypertension. A Cox proportional hazards model was used to explore the relationship between baseline blood lead and prospective incidence of hypertension. There was no significant change in the covariate adjusted risk of incidence of hypertension associated with baseline blood lead among this cohort. The authors note that the conversion of a continuous endpoint (BP) to a quantal endpoint (hypertension) could reduce the power of the longitudinal study to detect an effect. A significant positive association was reported for tibia lead and SBP in a cross-sectional analysis at baseline and a significant positive association was reported for patella lead and risk of hypertension in the longitudinal analysis. These results for bone lead are discussed in more detail in the section below on bone lead and BP.

Copenhagen County, Denmark

A cohort of 504 men and 548 women born in 1936 and residing in Copenhagen County, Denmark were followed from 1976 to 1981 (women) or 1987 (men). The longitudinal associations between blood lead and BP were first reported by Grandjean *et al.* (1989) and latter by Moller *et al.* (1992). Blood pressure was measured in 1976, 1981 and 1987 (men only) with a mercury sphygmomanometer. The measurements were taken with the subject in the supine position after 10 minutes of rest and the mean of 2 measurements was recorded. Nine potential confounding variables were considered and tobacco use, body mass index, physical activity, alcohol consumption and haemoglobin were retained in the final models. With the exception of a significantly positive cross-sectional association between women's blood lead and DBP, there were no significant relationships found between blood lead and BP after adjusting for confounding variables. A subsequent comment on the Grandjean *et al.* (1989) publication by Sharp (1990) provided evidence that blood lead could modify the hypertensive effect of alcohol and that controlling for alcohol intake could underestimate the possible relationship between blood lead and BP. Moller *et al.* (1992) agreed that adjusting for alcohol consumption could be problematic and also presents an argument that the blood lead-BP relationship should not be adjusted for haematocrit or haemoglobin.

Former Lead Workers, New Jersey USA

This cohort consists of middle aged, predominantly Caucasian (93%) males formerly occupationally lead exposed at a New Jersey chemical manufacturing facility. Although this is an occupationally exposed cohort, the mean length of time since occupational exposure was approximately 18 years and the mean blood lead and bone lead values among this formally occupationally exposed cohort is slightly less than that reported for the environmentally exposed older male adult subjects of the Normative Aging Study (NAS). The mean age of the NAS subjects was about 10 years older. Covariates include age, education, ethnicity, tobacco and alcohol use, body mass index, and use of hypertension medications.

Glenn et al. (2003) analyzed the longitudinal association between lead exposure and annual change in BP among 496 former and current male employees of a chemicalmanufacturing facility in the Eastern USA. The facility historically produced tetramethyl and tetraethyl lead. The average age at baseline was 55.8 (SD 7.4) years, the average time since occupational lead exposure was 17.7 (11.6) years and the average blood lead at baseline was 4.6 (2.6) µg/dL. Subjects were followed for an average of 2 (range 0.85-3.5) years and BP was assessed 3 or 4 times over this follow-up period. Sitting BP was measured using a random zero sphygmomanometer. BP was recorded as the average of three readings to the nearest 2 mmHg taken at five minute intervals. Generalized estimating equations (GEE) were used to evaluate the relationship between baseline blood lead and annual change in BP while controlling for other covariates. After adjusting for baseline age, body mass index, antihypertensive medications, smoking, education, technician and the time to each BP measurement, SBP increased at an average annual rate of 0.64 (SE: 0.25; 95% CI: 0.14-1.14) mmHg for every standard deviation (2.6 µg/dL) increase in baseline blood lead. This is equivalent to an adjusted linear dose-response slope of 0.25 mmHg per year per µg/dL. Significant positive associations were also reported between tibia lead and SBP and peak tibia lead and SBP. The results for bone lead are discussed in more detail below. No significant relationship between lead and DBP was found in this study.

Boston Police, Boston, USA

Weiss *et al.* (1986) report on the longitudinal relationship between blood lead and changes in BP among 70 middle-aged (mean age at baseline 46.6 years) male members of the Boston Police Department. Subjects were followed for 5 years and seated BP measurements were taken annually in triplicate with conventional sphygmomanometer. Blood lead was quantified in year two and subjects were divided into 3 exposure categories of approximately equal numbers: referent < 20

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 μ g/dL, low 20 to 30 μ g/dL, and high > 30 μ g/dL. Markov autoregressive analysis with fixed effects was performed to examine the relationship between blood lead and BP. After adjusting for previous systolic BP, body mass index, age and smoking, blood lead was found to be significantly positively associated with change in SBP for those with high blood lead (> 30 μ g/dL). No other statistically significant associations were reported.

"Foundry B", Canada

Neri *et al.* (1988) published a longitudinal analysis of blood lead and BP data from 288 employees at "Foundry B". Subjects appear to have been followed for 6 years. A significant positive association was reported between change in blood lead and change in DBP and a non-significant positive association was reported between change in blood lead and SBP. These results were corrected for age and weight. Very few details of this study were reported and the confounding effects of co-exposure to cadmium could not be adequately addressed. Therefore, the results of this study are not considered further.

Summary of Adult Blood Lead and BP

The collective epidemiological evidence of the relationship between blood lead and SBP among adults is difficult to interpret. On the one hand, multiple meta-analyses have concluded that there is a weak, but significantly positive association (Staessen *et al.* 1994a; Schwartz 1995; Nawrot *et al.* 2002). Three of six longitudinal studies published since 1980 that included SBP as an endpoint reported a significant positive association with blood lead after adjusting for covariates. Two of the three studies reporting a significant positive result were from currently occupationally exposed cohorts; the third positive study was from a formerly occupationally

comparable to environmentally exposed subjects at the time of the study. Additionally, the highest quality longitudinal study, one that included repeated blood lead measurements as well as careful conventional and 24 hour ambulatory measurements of SBP, failed to find a consistent association between blood lead and BP via multiple different analytical strategies (Staessen *et al.*, 1996). Comparisons among the available longitudinal studies are difficult because of differences in the populations studied (occupational, former occupational and environmental), lead exposure levels, the length of follow-up, the methods of measuring BP, the analytical models (effect of blood lead at baseline *vs* change in lead related to change in BP), and the extent to which known confounders were controlled for. The collective evidence from these studies is inconsistent, but supportive of an association between blood lead and increased SBP in adults.

Considered in isolation, it could be argued that the weight of evidence from epidemiological investigations of blood lead and SBP is inconclusive. However, one must also consider the totality of the evidence and also weigh the sensitivity of epidemiological studies of blood lead and BP to detect an effect before reaching and final conclusions on the plausibility that lead exposure can cause increased SBP in adult humans. The evidence from additional lines of inquiry, including that from epidemiological studies that used bone lead as an index of chronic lead exposure and data from animal assays and in vitro experiments, is stronger in support of an effect. There are also methodological limitations to the sensitivity of epidemiological studies of blood lead and BP to detect an effect. Measurement uncertainty exists for both blood lead and BP. The nondifferential classification that results from the low reliability of these measurements is null biasing and will underestimate their true associations. In summary, the lack of consistent positive results from epidemiological studies of the association between blood lead and BP can equally be interpreted as evidence of the limitations of existing epidemiological investigations to detect an association as they can be interpreted as evidence of an
absence of an association. The collective epidemiological evidence is inconsistent, but suggestive of an association between blood lead and SBP and the weight-ofevidence is judged sufficient to estimate a biomarker-response relationship between blood lead and increased SBP among humans that are chronically exposed to environmental lead. It is therefore recommended that a tolerable biomarker concentration (TBC) be developed for adult blood lead and SBP and that a critical study be selected from among the published epidemiological reports to establish a representative quantitative dose-response relationship between blood lead and SBP. In light of the variability in blood lead-SBP response relationships reported in the existing epidemiological literature, it is recommended that Health Canada review the adequacy of the adult TBC for blood lead to protect against unacceptable adverse population health effects within a period of no greater than 10 years from the date of publication of this document.

Bone Lead and SBP in Adults

Blood lead, particularly a single blood lead measurement, as has often been used as an estimate of lead exposure in the epidemiological studies of lead exposure. While blood lead measurements are relatively precise, inexpensive, easy to collect and have analytical methods that are well established and standardized, a blood lead measurement is not the best measure of chronic lead exposure. Lead in the blood reflects recent and historical exposure and the relative dominance of each is a function of the pattern of exposure. While the pathophysiology of lead-induced hypertension is not understood completely, there is evidence from *in vivo* and *in virtro* studies that suggests that there may be multiple mechanisms of action whereby lead can induce hypertension and some of these are chronic effects. For example; when rats with chronic lead-induced hypertension are treated with antioxidant chelation therapy their blood lead returns to control levels but their BP, while diminished, does not return to control levels (Ding *et al.*, 1998). A similar pattern was observed in a stop exposure study (Chang *et al.*, 2005). This suggests that there is a persistent effect, or at least a delayed repair mechanism that could make blood lead and BP asynchronous. If this is the case, a measure of chronic or cumulative lead exposure would be better correlated with BP than the hybrid index of exposure provided by a single blood lead.

Two alternate methods to a single blood lead measurement that provide a better estimate of chronic lead exposure are: (1) cumulative blood lead index (CBLI); and (2) bone lead measurements. No published studies of the association between CBLI and BP were located at the time of this report and this method, therefore, will not be discussed here as lead accumulates in bone and bone lead concentrations are a better biomarker of chronic lead exposure than a single blood lead or blood lead sampled over a relatively short period of the subject's life span. This issue was recently reviewed by Hu et al. (2007). While in vivo bone lead measurements using x-ray fluorescence (XRF) methods have been available for about 30 years (Chettle, 2005), most of the epidemiological studies using bone lead as a biomarker of chronic lead exposure have been published within the last decade. The available studies of bone lead and BP are reviewed below. As a whole, the bone lead studies provide more consistent results, which should be expected with a better biomarker of chronic lead exposure. This appears to also be true for other chronic health endpoints, such as neurological effects in adults (Shih et al., 2007). Despite the encouraging developments in *in vivo* bone lead measurement and the consequent improvement in estimating chronic lead exposure among epidemiological study subjects, XRF bone lead measurements, while improving, are relatively imprecise particularly at low levels of exposure. This imprecision of measurement adds to the noise in the exposure-effect relationship and will be null biasing. Additionally, interlaboratory standards have yet to be fully developed for XRF bone lead measurements and inter-study comparisons are, therefore, potentially limited by this issue.

Bone Lead and SBP

As discussed above, bone lead measurements offer a potentially superior index of chronic lead exposure (Chettle, 2005; Hu *et al.*, 2007). To the extent that effects on BP caused by environmental lead exposures are chronic, epidemiological investigations that estimate lead exposure via a bone lead measurement can potentially provide a better indication of the chronic exposure-response relationship than epidemiological studies that employ a single or limited serial blood lead measurement.

There are 11 publications from six cohort studies on the association of bone lead and BP or risk of hypertension (Hu *et al.*, 1996; Korrick *et al.*, 1999; Schwartz and Stewart, 2000; Cheng *et al.*, 2001; Lee *et al.*, 2001; Gerr *et al.*, 2002; Rothenberg *et al.*, 2002a; Glenn *et al.*, 2003; Glenn *et al.*, 2006; Martin *et al.*, 2006). A metaanalysis on 8 of these studies has also been conducted (Navas-Acien *et al.*, 2008). A summary of the results of these studies is presented in Table 14.

 Table 14. Summary of epidemiological studies of bone lead and blood pressure or risk of hypertension.

 Type of study: XS, cross-sectional; L, longitudinal. Results, after adjusting for covariates: +, significant

 positive association; 0, no significant association; -, significant negative association. N/R: not reported.

Reference	Cohort	Туре	Cortical Pb			Trabecular Pb		
Occupational			SBP	DBP	HYP	SBP	DBP	HYP
Lee <i>et al.</i> 2001	S. Korean Pb workers	XS	0	0	+	N/R	N/R	N/R
Glenn <i>et al.</i> 2006	S. Korean Pb workers	L	-	N/R	N/R	N/R	N/R	N/R
Schwartz and Stewart 2000	Former organolead workers, US	XS	0	0	0	N/R	N/R	N/R
Glenn <i>et al.</i> 2003	Former organolead workers, US	L	+	0	N/R	N/R	N/R	N/R
Hu <i>et al.</i> 1994	Int'l. Brotherhood of Carpenters & Joiners	XS	0	0	0	0	0	0
Environmental								
Rothenberg <i>et al.</i> 2002	Pregnant ♀, LA, US 3 rd trimester	XS	0	0	0	+	+	+
	Pregnant ♀, LA, US postpartum	XS	0	0	0	0	0	0
Hu <i>et al.</i> 1996	NAS, Boston, US	XS	N/R	N/R	0	N/R	N/R	0
Cheng <i>et al.</i> 2001	NAS, Boston, US	XS	+	N/R	N/R	0	N/R	N/R
Cheng <i>et al.</i> 2001	NAS, Boston, US	L	N/R	N/R	0	N/R	N/R	+
Korrick <i>et al.</i> 1999	Boston Nurses' Health Study, US	XS	N/R	N/R	0	N/R	N/R	+
Gerr <i>et al.</i> 2002	Bunker Hill/Spokane, US	XS	+	+	N/R	N/R	N/R	N/R
Martin <i>et al.</i> 2006	Baltimore Memory Study, US	XS	0	0	0	N/R	N/R	NR

The endpoint that has been most commonly reported on is SBP. Systolic blood pressure is also the toxicological endpoint that was selected as the critical effect for adult blood lead. For these reasons the emphasis here will be on reviewing the evidence of an association between bone lead and SBP. There is only one published report on the relationship between trabecular bone and SBP (Rothenberg *et al.*, 2002a). Therefore, this review will focus on the relationship between cortical bone lead, as measured in the tibia, and SBP.

The relationship between tibia lead and SBP has been examined in seven cohorts, of which three were occupational and four were environmental (see Table 14). A

significant positive association, after adjusting for covariates, was reported in three of the seven cohorts. Three of the six found no significant association. A significant negative association was found in the longitudinal analysis of the South Korean lead workers. The absence of reporting for this endpoint in some of the studies raises suspicions of publication bias. No clear trend is evident with respect to results and occupational or environmental exposures, although the contemporary blood and bone lead concentrations in the former organolead workers is comparable to those found in the environmental cohorts. The collective evidence is inconsistent, but suggestive of an association between tibia lead and SBP. The details of the individual studies are provided below.

Meta-Analysis

Navas-Acien et al. (2008) conducted a systematic review and meta-analysis of studies published prior to May 2007 on the association of bone lead and blood pressure or risk of hypertension. Ten studies were identified, but two were excluded from the meta-analysis as the original studies corrected the bone lead to outcome relationships for blood lead. Tibia lead was most commonly used as a measure of lead exposure and was reported for six studies (two longitudinal and four crosssectional). Two of the six studies included in the meta-analysis reported a statistically significant positive association between tibia lead and SBP and tibia lead and risk of hypertension. Using an inverse variance weighted random-effects model, the meta-analysis of the association between tibia lead and SBP and tibia lead and risk of hypertension across the four cross-sectional studies were positive and statistically significant. A positive, but not significant relationship was also reported for cross-sectional tibia lead and DBP (three studies) and longitudinal tiba lead and SBP (two studies). The authors concluded that SBP and risk of hypertension were positively associated with tibia lead but the magnitude of the summary estimate of effect was small.

Occupational Exposed Cohorts

Five publications on three different cohorts have been completed to examine the effects of bone lead on BP or hypertension from occupational exposure. Lee *et al.* (2001) conducted a cross-sectional analysis of current lead workers in South Korea and Glenn *et al.* (2006) conducted a longitudinal analysis of this same cohort. A cohort of former organolead workers from New Jersey, USA was examined in both cross-sectional (Schwartz and Stewart, 2000) and longitudinal studies (Glenn *et al.*, 2003). Hu *et al.* (1994) conducted a cross-sectional analysis of bone lead and BP and hypertension among 119 members of the International Brotherhood of Carpenters and Joiners.

South Korean Lead Workers

Lee *et al.* (2001) and Glenn *et al.* (2006) have reported on the relationship between lead biomarkers and BP and hypertension among an occupational cohort employed at 26 lead-using facilities in South Korea. The cohort is 80% male with an average age at baseline (1997) of 40.5 (SD: 10.1) years. The average duration of employment in lead exposed occupations was 8.5 years. The bone and blood lead concentrations in the South Korean lead workers were much higher than those reported for all of the other cohorts. The mean tibia lead among this cohort, 38.4 (40.8) μ g/g is approximately double that in the Normative Aging Study (NAS) cohort (21.9 (13.5) μ g/g) and an order of magnitude greater than that in the Bunker Hill/Spokane cohort (4.2 (NR) μ g/g). Triplicate seated blood pressure measurements were made using a mercury sphygmomanometer at 5 minute intervals. Data was also collected on the following covariates: age, education, hypertension status, body mass index, smoking status, alcohol status, and job duration. Lee *et al.* (2001) published a cross-sectional study of the South Korean lead workers and a referent cohort with no occupational lead exposure. Linear regression analysis was used to explore the cross-sectional relationship between lead dose and BP and hypertension. Among 798 workers, dimercaptosuccinic acid (DMSA)-chelatable lead was significantly positively associated with SBP. A non-significant (P>0.05) positive association was also reported for tibia lead and blood lead and SBP. None of the measures of lead exposure were significantly related to DBP. Tibia lead only was positively associated with risk of hypertension.

More recently, Glenn *et al.* (2006) conducted a longitudinal analysis of this cohort to try and determine whether the effect of lead on BP is acute or chronic. Multiple measures of blood lead, tibia lead and BP were obtained from 575 subjects over 3 years. Longitudinal data were analyzed in multivariate models using generalized estimating equations (GEE). Four models were constructed to explore possible relationships between acute and chronic lead exposure (represented by blood lead and tibia lead, respectfully) and change in SBP. A significant positive longitudinal association was reported, after adjusting for covariates, between blood lead and SBP. The magnitude of effect (β (95%CI)) of annual increase in SBP was approximately 0.1 (0.01-0.19) mmHg per µg/dL for both cross-sectional blood lead and longitudinal blood lead. A significant negative longitudinal relationship was reported between tibia lead and SBP.

Former New Jersey Organolead Workers

Schwartz *et al.* (2000) and Glenn *et al.* (2003) have conducted a cross-sectional and longitudinal analysis, respectfully, on a cohort of male, middle-aged, primarily Caucasian (93%) former organolead-manufacturing workers from New Jersey state, USA. Occupational exposure had taken place an average of 18 years prior to the study and the mean tibia lead among this cohort (14.7 (9.4) μ g/g) is less than that of

the similarly aged environmental cohort of men participating in the NAS (21.9 (13.5) μ g/g). Triplicate seated blood pressure measurements were made using a mercury sphygmomanometer at 5 minute intervals. Data was also collected on the following covariates: age, ethnicity, education, diabetes, body mass index, smoking, and alcohol use.

Schwartz *et al.* (2000) performed a cross-sectional study of 543 subjects of this cohort. The authors examined relationships among blood lead, tibia lead, and dimercaptosuccinic acid (DMSA)-chelatable lead, BP and prevalence of hypertension. Blood lead, after adjusting for covariates, was associated with SBP, DBP and hypertension amongst men < 58 years old. (DMSA)-chelatable lead and tibia lead were not associated with BP or hypertension.

Glenn *et al.* (2003) conducted a longitudinal analysis on 496 subjects from this cohort who had three or four repeat BP measurements. The average length of follow-up was 2 years and the effect of lead on the annual change in BP was evaluated using generalized estimating equation (GEE) models. The results indicated that blood lead, concurrent tibia lead and (modeled) peak tibia lead showed significant positive association with increasing SBP, with tibia lead having the greatest effect size. The magnitude of effect (β (95%CI)) of annual increase in SBP was approximately 0.078 (0.024-0.13) mmHg per µg/g. Blood lead was associated with DBP at baseline, but no longitudinal association with changing DBP was observed. Concurrent tibia lead and (modeled) peak tibia lead showed weaker cross-sectional association with DBP at baseline and no longitudinal association.

Although this is presented as a longitudinal study, the measures of lead exposure only occurred at a single point in time. Health endpoints (SBP and DBP) were followed for changes over time. The results of Glenn *et al.* (2003) were not able to elucidate whether BP effects are chronic or acute. Neither baseline blood lead nor peak tibia lead were dominant predictors of BP when included in the same model. Glenn *et al.* (2003) were limited in this regard by a single baseline blood lead measurement. They suggest that the chronic or acute origins of PB effects could be better examined with a study design that includes multiple synoptic measures of blood lead and BP.

International Brotherhood of Carpenters and Joiners

Hu et al. (1994) conducted a cross-sectional analysis of bone lead and BP and hypertension among 119 members of the International Brotherhood of Carpenters and Joiners. The place of residence and occupation of these subjects is not reported, but because measurements were made on a sample of attendees of the US national conference of the International Brotherhood of Carpenters and Joiners, subjects were primarily middle-aged male Caucasians from the US. Subjects were employed in a number of construction-related trades that could result in occupational lead exposure. A single blood pressure measurement was made using a mercury sphygmomanometer. X-ray fluorescence bone lead measurements were taken over 12.5 minutes – much shorter than the 30 minute duration of measurement employed in all other studies summarized in this document. Data was also collected on the following covariates: age, body mass index, smoking, alcohol use, and use of hypertensive medication. The results of multiple linear regression analysis indicated that neither tibia lead, patella lead, nor blood lead were significantly associated with BP or hypertension. The study authors note that the negative results could be a reflection of the small sample size and the measurement error associated with an abbreviated XRF bone lead measurement and a single BP measurement.

Environmentally Exposed Cohorts

Veteran Affairs Normative Aging Study

Participants in the Veteran Affairs Normative Aging Study (NAS) from greater Boston, MA have been subject to both cross-sectional (Hu *et al.*, 1996) and longitudinal (Cheng *et al.*, 2001) study. The subjects are middle-aged to elderly, predominantly Caucasian men. Blood pressure was measured as the mean of conventional seated sphygmomanometer readings from each arm. Possible confounding variables included age, age squared, body mass index, family history of hypertension, ethnicity, education, smoking, alcohol use, and dietary intake of Na and Ca. In this study, hypertension was defined as a mean SBP> 160 mmHg, DBP > 95 mmHg, or taking daily medication for the treatment of hypertension.

Hu *et al.* (1996) conducted a cross-sectional analysis of the association between bone lead and risk of hypertension among 590 subjects in the NAS. Tibia lead and calcaneous lead were associated with a non-significant increase in odds ratio (OR) for hypertension. BP was not included as study endpoint in this publication.

Cheng *et al.* (2001) published a cross-sectional and longitudinal study of the same cohort as Hu *et al.* (1996), but examined BP as a continuous endpoint for the cross-sectional analysis. In the cross-sectional analysis of 519 subjects who were normotensive at baseline tibia lead was significantly positively associated with SBP. A non-significant postitive association was also reported for patella lead and SBP. Effects on DBP were not reported. Longitudinal data were available for 474 subjects who were followed for approximately 3 years. Patella lead, but not tibia lead nor blood lead were also associated with prospective incidence of hypertension. The rate ratio for incidence of hypertension was stronger for normotensives at baseline.

After controlling for the confounding influence of demographic and behavioural risk factors, the dose-response relationship between bone lead (tibia and patella) and

risk of hypertension was approximately 2½ fold higher among subjects of the Normative Aging Study who self-reported high stress (Peters *et al.*, 2007).

Nurses' Health Study

Korrick *et al.* (1999) conducted a case-control study of 284 (89 cases, 195 controls) of middle-aged to elderly women from Boston MA participating in the Nurses' Health Study for associations between blood, tibia and patella lead and risk of hypertension. Triplicate seated blood pressure measurements were made using a mercury sphygmomanometer at 5 minute intervals. Data was collected on the following covariates: age, body mass index, alcohol use, smoking, family history of hypertension and dietary sodium and calcium. Results indicated a significant positive association between patella lead and risk of hypertension, but none for tibia lead and blood lead.

Bunker Hill, ID & Spokane, WA

Gerr *et al.* (2002) performed a cross-sectional study of 508 young adults from western US. Subjects were either childhood residents from communities surrounding the Bunker Hill, ID lead smelter who were heavily lead exposed as children or from an age-matched referent cohort residing in Spokane, WA at the time of the study. The subjects were mixed sex, Caucasian, young adults, 19 to 29 years old at the time of the study. A single seated blood pressure measurement was made using a mercury sphygmomanometer. Data was collected on the following covariates: age, sex, height, body mass index, smoking, alcohol consumption, use of oral contraceptives, blood haemoglobin, childhood residence, and income. The relationships between tibia lead, BP and covariates was analyzed using multiple linear regression with bone lead defined as a four-level ordinal variable. Systolic blood pressure and DBP were significantly positively associated with the highest

tibia lead exposure group (tibia lead > 10 μ g/g) after adjusting for covariates. The study authors were contacted but the parameters for the continuous tibia lead regression model, which the authors reported as significantly and positively associated with SBP, but they were not obtained (McNeill, 2007).

Latinas in Los Angeles, CA

Rothenberg et al. (2002) completed a cross-sectional study of associations between blood and bone lead and hypertension and BP at 3rd trimester and postpartum among 1,006 primarily Latina (81%) immigrant women enrolled in Los Angeles prenatal care clinics. Postpartum assessments were performed at an average of 10 weeks (max: 18 weeks) after delivery. Triplicate seated blood pressure measurements were made using an automatic device at 3 minute intervals following 30 minutes of rest. Covariates examined included: nursing (postpartum), smoking, alcohol consumption, ethnicity, immigration status, age, education, income, and body mass index. After adjusting for covariates, the authors reported a significant positive association between calcaneus lead and 3rd trimester hypertension, SBP and DBP. Calcaneus lead was not associated with BP or hypertension postpartum. and tibia lead and blood lead were not associated with BP or hypertension at either assessment period. The authors also reported significant negative association between postnatal blood lead and postnatal SBP and DBP, although they note that this observation was made during the period of normal postpartum changes in cardiovascular parameters.

Baltimore Memory Study

The Baltimore Memory Study is a longitudinal study of the determinants of cognitive decline in urban residents of Baltimore, Maryland. Martin *et al.* (2006) conducted a cross-sectional analysis of the associations between tibia lead and blood lead with

BP and hypertension among 964 multi-ethnic (55% Caucasian, 40% African-American, 5% other), mixed sex (66% women), older (mean age 59 years) subjects. Environmental lead exposures were relatively low in this cohort; the mean blood lead of subjects was 3.5 (SD: 2.3) µg/dL. Triplicate seated blood pressure measurements were made using a mercury sphygmomanometer. Data was collected on the following covariates: age, sex, body mass index, use of antihypertensive medication, dietary sodium and calcium, time of day of measurement, testing technician, serum cholesterol and homocysteine, household wealth, educational status, occupational status and ethnicity. Multiple linear regression analysis was used to examine the relations of blood lead and tibia lead with BP. After adjusting for covariates, there was a significant positive association between blood lead and BP, but there was no significant association between tibia lead and BP. Multiple logistic regression was used to examine associations of blood and tibia lead with hypertension status. There was a non-significant (p=0.09) positive association between tibia lead and hypertension status, after adjusting for covariates. There was also no significant association between blood lead and hypertension status. The authors of the study also undertook a propensity score analysis – the results of which suggests that controlling for SES and ethnicity in multiple regression analysis may underestimate the overall effect of lead exposure on BP and hypertension.

Discussion and Conclusions

Associations of tibia lead and SBP (as well as SBP and risk of hypertension) are not consistent in the available epidemiological studies. The relationship between tibia lead and SBP has been examined in a total of seven cohorts - three occupational and four environmental. A significant positive association, after adjusting for covariates, has been found in three of the seven cohorts. Three of the six found no significant association. And a significant negative association was found in the longitudinal analysis of the South Korean lead workers. The absence of reporting for this endpoint in some of the studies raises suspicions of publication bias. No clear trend is evident with respect to results from occupational or environmental cohorts.

The existing epidemiological evidence has several limitations which could bias conclusions drawn from this body of evidence in both the false positive as well as the false negative direction. It remains plausible that the significant positive association between tibia lead and SBP reported in some studies could be an artefact of residual confounding. Important determinants of BP that could also be positively correlated with lead exposure have not always been investigated; these include exposure to noise and dietary sodium and potassium.

On the other hand controlling for SES and education, which may both influence as well as be influenced by lead exposure, could lead to underestimation of the lead effect. Measurement error associated with XRF bone lead measurement and BP measurement is also null-biasing. Finally, effect modification by variables such as genetic variability has also not been taken into account and can add to the noise in the biomarker-response relationship. Stratification by genotype can have a remarkable effect on the ability to detect a relationship between bone lead effects and other health endpoints (Wang *et al.*, 2007).

There is also very strong evidence from animal experiments and well demonstrated mechanisms that establish that chronic lead exposure can cause increased blood pressure in mammals. Significant positive associations between blood lead and BP and hypertension have also been reported in many, but not all, epidemiological investigations. Yet none of the authors of the studies that reported a nil or a negative association between tibia lead and BP have offered a plausible biological explanation in support of their observational results.

On balance the reported nil associations between tibia lead and SBP can equally be interpreted as evidence of the limitations of existing epidemiological investigations to detect an association as they can be interpreted as evidence of an absence of an association. The collective epidemiological evidence, while inconsistent, is suggestive of an association between tibia lead and SBP. There is strong supporting evidence from other lines of inquiry that chronic lead exposure causes increased blood pressure in mammals and there is, to-date, no plausible explanation for why there should not be a biomarker-response relationship between tibia lead and SBP as a result of chronic lead exposure.

Epidemiological Evidence of BP Effects in Children

There is currently insufficient evidence to derive a TRV on the basis of BP effects of lead in children. Only 1 of 5 studies published before the mid 1990's on the relationship between children's (< 10 years of age) blood lead and BP reported a significant positive association. These studies are not considered further, however, because they all had relatively few subjects (<150) and did not adequately control for confounding. Studies of the association between children's blood lead and BP were also excluded from the Nawrot et al. (2002) meta-analysis. Therefore, the only epidemiological evidence of the association between blood lead and BP that was considered in detail are the 3 recent prospective epidemiological studies of the relationship between lead exposure and BP in children. Two of the three studies (Factor-Litvak et al., 1996; Chen et al., 2006) report no significant effect of blood lead and BP. Gump et al. (2005) report a positive association between cord blood lead, but not early childhood blood lead, and resting BP in children at 9.5 years of age. However, Gump et al. (2005) also report changes in stress-induced cardiovascular function at 9.5 years of age associated with early childhood blood lead that could be indicative of lead-related cardiovascular effects that could lead to latter life development of hypertension and other cardiovascular morbidity and

mortality. At the time of this report, no studies on the relationship between bone lead and BP in children were located in the published literature.

Considered collectively, the current epidemiological evidence is insufficient to support an association between lead exposure and increased BP in children. The recently reported results from the Oswego Children's Study (Gump *et al.*, 2005) suggest that early life lead exposure can result in cardiovascular effects that may initially have little net effect on BP, but which may ultimately lead to hypertension or other cardiovascular morbidity and mortality. This line of reasoning, however, requires additional supporting evidence.

Multi-centre Treatment of Lead-Exposed Children Trial

The Treatment of Lead-Exposed Children (TLC) trial is a randomized placebocontrolled, double blind, multicentre clinical trial to test the efficacy of chelation therapy in mitigating cognitive and behavioural effects of lead in toddlers with moderately high blood lead concentrations (20-44 µg/dL). Subjects were enrolled from 12 to 33 months of age and followed for 5 years post treatment. Sevetny-seven percent of the children in this study were African-American, 5 % were Hispanic, and the balance were Caucasian. The effects of chelation-induced changes in BP as well as a cross-sectional analysis of the association between blood lead and BP among 780 subjects of the TLC were reported in Chen *et al.* (2006). Subjects received up to three 26-day treatments with either oral dimercaptosuccinic acid (succimer) or a placebo. Blood lead and BP were measured at baseline and at 7, 28, and 42 days following initiation of each round of treatment and at three to four month intervals thereafter for five years of follow-up. BP was measured with an automatic device and the average of up to three seated measurements was used for statistical modeling. Various statistical analysis were preformed to explore the relationships among treatment group, lead exposure, and BP. Multiple regression models were used to test the hypothesis that succimer treatment lowered BP. Multiple regression modeling was also used to test the covariate adjusted cross-sectional relationships at various time points between concurrent blood lead and BP. Finally, mixed regression models were used to account for the repeated measurement of BP and explore the temporal relationship between blood lead and BP. Covariates examined included clinical centre, baseline blood lead, ethnicity, sex, parental education, single parent, age, height and body mass index.

The average blood lead of subjects declined from 26(SD: 5) µg/dL to 8(4) µg/dL over the five year study period. Children treated with succimer experienced a transient decline in blood lead relative to the placebo treatment group. The succimer induced reduction in blood lead lasted for nine to ten months. Succimer treatment did not change BP from initiation to nine months follow-up. Over the interval of one year to five years post treatment, the succimer group had a 1 mmHg increase in SBP relative to the placebo group. Analysis with the mixed models as well as cross-sectional analysis revealed no consistent association between blood lead and BP. An example of the lack of an association between concurrent blood lead and BP is illustrated in Figure 8 below. Overall this study found no association between blood lead and BP.

TOXICOLOGICAL REVIEW AND RECOMMENDED TRVS FOR ENVIRONMENTAL LEAD EXPOSURE IN CANADA



Figure 8. Reproduced from Chen et al. (2006). Scatter plot of systolic and diastolic blood pressure by concurrent blood lead concentration at 60 months follow-up. Solid lines are unadjusted spline regression estimates and dashed lines are their 95% CI's.

The Chen *et al.* (2006) study has, of all the published studies to examine the effects of lead exposure on children's BP, the largest sample size and also includes a repeated measurements of blood lead and BP. This gives it the greatest power to detect an effect; however, as the authors note, even their study only had power of 0.8 to detect up to a 2 mmHg change in BP between treatment groups for a given cross-sectional comparison. The comparisons at multiple time points make this less of a concern.

The results of the Chen *et al.* (2006) study are somewhat at odds with those from experimental animal studies, where two week treatment with 0.5% DMSA in the

drinking water of mature, male rats with lead-induced hypertension resulted in a significant decrease in BP (Ding et al., 1998). This inconsistency, however, may be due to differences in dose between the two studies. The children in the TLC trial were orally dosed with 1050 mg/m^2 body surface area succimer (DMSA) for 1 week and 700 mg/m² body surface area thereafter (Rogan et al., 2001). Based on the reported treatment duration of 3 weeks, this is equivalent to an average daily dose of 817 mg/m² body surface area. Based on Health Canada's default body area (approximately 3000 cm²) and weight (17 kg) for a toddler (Health Canada, 2004), 817 mg/m² body surface area is approximately equivalent to a dose of 14 mg/kg. Assuming the rats in the (Ding et al. 1998) study weighed 200 g and consumed 1 ml of water per day, the rats received an oral dose of approximately 250 mg/kg DMSA at least one order of magnitude higher than the oral dose the children in the TLC trial received. The DMSA dose administered in the TLC trial may not have been high enough for the antioxidant effects of DMSA to be effective at lowering the subject's BP. If the hypertensive effects of lead are persistent, the transient decrease in blood lead of the children in the TLC trial may not have been of sufficient duration or magnitude to allow BP to recover. Another explanation for the divergent results is that lead did not result in any hypertensive effect in the children of the TLC trial. This interpretation is consistent with the results of the cross-sectional analysis of the TLC data.

Yugoslavia Prospective Study of Environmental Lead Exposure

Subjects for the Yugoslavia Prospective Study of Environmental Lead Exposure were recruited from 2 towns in Kosovo, former Yugoslavia: Kosovska Mitrovica (Mitrovica) is host to a lead refinery, smelter, and battery factory; Pristina is a town 25 miles to the south with no significant point sources of lead exposure (lead paint was banned in Yugoslavia in 1922 and automobile traffic was minimal). Because of this unique approach to subject recruitment, the Yugoslavia study has the widest range of lead exposure (children's blood lead concentrations ranged from 1-70 μg/dL) and socioeconomic status of all the children's prospective lead study cohorts. 706 mother-infant pairs were recruited in 1985 and 1986. Assessment of health outcomes predated the hostilities between Serbia and Kosovo.

Maternal blood lead was sampled at mid-pregnancy and delivery (cord). Postnatal blood lead (venous) was sampled semi-annually from birth until 7.5 years of age. BP was measured at 5 years of age with an automatic device. Six measurements were made; three at the beginning of the assessment appointment and 3 at the end of the appointment. The mean of the last 2 measurements was used to represent blood pressure. A wide range of medical, biological, socioeconomic and environmental covariates were examined, including nutritional status. Socioeconomic status (SES) was not directly measured, but ethnicity (Albanian, Serbian or other) was thought to provide a reasonable surrogate for SES. Exclusion criteria included gestation <28 weeks or >44 weeks, multiple births, residence more than 10 km from the community pediatric center, and those with chromosomal abnormalities or nervous system defects.

Results of the investigation of the association between blood lead and BP among 282 children in the Yugoslavia Prospective Study of Environmental Lead Exposure are reported in Factor-Litvak *et al.*, 1996 and Factor-Litvak *et al.*, 1999. Data from both communities were combined and analyzed with multiple linear regression. After correcting for height, body mass index, waist circumference, sex, ethnicity and birth order, a positive, but non-significant cross-sectional association between blood lead and BP was reported.

Oswego Children's Study

Gump *et al.* (2005) report on the association between cord lead and early childhood blood lead and cardiovascular functioning at rest and in response to acute stress in children at 9.5 years of age. The subjects of this study were 122 children (46% male, ethnicity not reported) enrolled in the Oswego Children's Study, a prospective birth cohort study to examine the effects of prenatal PCB exposure on children's development. Mean cord blood lead was 1.98 (SD: 1.75) μ g/dL. Early childhood blood lead data was derived from state mandatory pre-kindergarten screening records. The average age early childhood blood lead was 4.62 (SD: 2.51) μ g/dL. This study examined a wide range of potential confounding variables. Data on covariates was collected during pregnancy, at birth and at 7.5 and 9.5 years of age. Covariates included maternal and subject morphological parameters, pregnancy and birth parameters, obstetrical complications, maternal substance abuse during pregnancy, Home Observation for Measurement of the Environment (HOME) score, maternal depression, and SES.

Cardiovascular functioning was assessed at 9.5 years of age. Subjects were required to complete a mirror tracing task and choice reaction time task to produce α -andrenergic and β -andrenergic cardiovascular responses, respectively. Cardiovascular function was monitored prior to and during the stress related tasks. Blood pressure was monitored with an automated arm cuff. Stroke volume and heart rate were measured by non-invasive impedance cardiography and electrocardiogram. Total peripheral vascular resistance (TPR) was calculated as a function of BP, stroke volume and heart rate. Cord blood lead, but not childhood blood lead, was reported to be significantly positively associated with resting BP at 9.5 years of age. There was also very little net effect on BP in response to acute stress. However, there was a significant increase in TPR in response to acute stress associated with increasing early childhood blood lead. These data are presented in Figure 9. Concurrent reductions in stroke volume and cardiac output counteracted the increased TPR and resulted in very little net effect on BP response to acute stress. Reduced stroke volume and cardiac output suggests that the increased TPR was sufficient to produce cardiac afterload – when aortic BP reduces the ability of the left ventricle to eject blood. There was no significant effect of sex on these results. Neither did excluding 6 subjects with blood lead > 10 μ g/dL significantly affect the results. The addition of quadratic or cubic terms to the regression model did not significantly improve the model fit – indicating that there was no clear threshold for the TPR effects over the range of early childhood blood lead concentrations measured in this study.

The results of this study are significant in that they suggest that early life lead exposure could contribute to the development of hypertension and cardiovascular morbidity and mortality later in life. This is currently the only epidemiological study located that examines the relationship between prenatal blood lead and BP in childhood – and reports a significant positive association. The lack of association between postnatal blood lead and BP is consistent with the few other epidemiological studies of this relationship in children, which have been consistently negative (Factor-Litvak et al., 1996; Chen et al., 2006). However, this study suggests that haemodynamic effects, such as reduced cardiac output, may initially counteract the effects of increased vascular resistance, resulting in little net effect on BP. Increased vascular reactivity, however, could eventually lead to the development of hypertension and other cardiovascular morbidity and mortality. There is evidence that increased TPR leads to higher blood pressure and ventricular hypertrophy which, aside from age, is the strongest known predictor of cardiovascular morbidity and mortality. Increased TPR can lead to hypertension either directly, through structural remodeling or resistance vessels or thickening of vascular walls, or indirectly via the development of atherosclerosis.



Figure 9. Reproduced from Gump et al. (2005). Change to total peripheral vascular resistance (TPR) (dyns/cm5) in response to acute stress at 9.5 years of age as a function of early childhood blood lead (µg/dL). Adjusted for maternal pre-pregnancy weight, weight gain during pregnancy, stress before and during pregnancy, maternal history, prescription medications, depression, and nutrition; cord blood lead; and subject sex, gestational age and obstetrical complications.

Children, Conclusions

There is currently insufficient evidence to derive a TRV on the basis of BP effects of lead in children. Only one of five studies published before the mid 1990s on the relationship between children's (< 10 years of age) blood lead and BP reported a significant positive association. Two of the three recent prospective epidemiological studies (Factor-Litvak *et al.*, 1996; Chen *et al.*, 2006) report no significant effect of blood lead and BP. The recently reported results from the Oswego Children's Study

(Gump *et al.*, 2005) suggest that early life lead exposure can result in cardiovascular effects that may initially have little net effect on BP, but which may ultimately lead to hypertension or other cardiovascular morbidity and mortality. This line of reasoning, however, requires additional supporting evidence. At the time of this report, no published studies on the relationship between bone lead and BP in children were located in the literature. Considered collectively, the current epidemiological evidence is insufficient to support an association between lead exposure and increased BP in children.

4.3 EVIDENCE OF VASCULAR TOXICITY FROM ANIMAL EXPERIMENTS

There is very strong evidence that chronic and subchronic lead exposure causes increased BP in animals even at relatively low, environmentally relevant levels of exposure. The evidence of the vascular toxicity of lead has been recently reviewed and summarized by numerous authors (Staessen *et al.*, 1994b; Vaziri and Sica, 2004; IARC, 2006; US EPA, 2006; Vaziri and Khan, 2007; Vaziri, 2008). Earlier reviews by Victery (1988) and Staessen *et al.* (1994b) were also consulted and provide excellent summaries of some of the older literature on this subject, which has been studied in animal laboratories for at least the last 50 years.

While the early literature on the vascular toxicity of lead reported inconsistent results and has been criticized for methodological issues (Atchison, 2007), there are many recent, well designed published studies that consistently report that chronic environmentally relevant exposure of laboratory animals to lead result in significant elevations in blood pressure. Lead exposure has been shown to result in increased BP in animal experiments with rats, dogs (Fine *et al.*, 1988) and pigeons (Revis *et al.*, 1981). The US EPA, in a recent, peer reviewed and comprehensive survey of the literature in support of the National Ambient Air Quality Standard for lead, stated that there was "irrefutable evidence that extended exposure to low levels of lead can

result in the subsequent onset of HTN (hypertension) in (genetically normal) experimental animals" (US EPA, 2006).

The methodological criticisms of earlier studies include the stress placed on animals by tail-cuff BP measurements, the use of genetically susceptible stains, and that the observed vascular effects were secondary to the nephrotoxic effects of lead. All of these issues have been addressed and overcome in the more recent generation of studies. With respect to the inconsistency of results from earlier studies, one of the curious patterns that emerges when looking at the collective evidence from *in vivo* studies is that adverse effects on BP are consistently reported at relatively low dose exposures, whereas some of the relatively higher dose exposures are not reported to be associated with a statistically significant effect. While some of this inconsistency may be attributed to between study variation, the same pattern has been reported within studies conducted under the same experimental conditions. A detailed characterization of this dose-response relationship has not been developed, nor has a satisfactory explanation for its presence been offered. Nonetheless, a positive effect on blood pressure has been consistently reported for the range of lead exposures which are typical of contemporary environmental exposures.

The redundancy of summarizing all of the available animal studies on lead and blood pressure was considered unnecessary. There have been at least 25 published studies on this subject (see Staessen *et al.* (1994b) for a summary of those published between 1977 and 1998). A selection of more recent, high quality studies are presented in the Key Studies section below. In isolation, these studies provide very strong *in vivo* experimental evidence of the hypertensive effects of lead. The reader is reminded that there are many additional studies that support the conclusions of those summarized here.

Key Studies

Chronic experimental exposure of mature male laboratory rats to 100 lead (as lead acetate) in drinking water has repeatedly reported a significant increase in blood pressure (BP) associated with blood lead concentrations as low as 2.4 \pm 0.6 µg/dL (Attri *et al.*, 2003). Significant increases in BP have been observed at this exposure level following as little as eight weeks of exposure (Ding *et al.*, 1998).

There have been at least seven published laboratory studies reporting a statistically significant increase in BP in rats sub-chronically or chronically exposed to lead in drinking water with blood lead concentrations < 20 μ g/dL (Khalil-Manesh *et al.*, 1994; Gonick *et al.*, 1997; Vaziri *et al.*, 1997; Ding *et al.*, 1998; Vaziri *et al.*, 1999a; Vaziri *et al.*, 1999b; Ding *et al.*, 2001; Attri *et al.*, 2003). Of these studies, four reported a statistically significant increase in BP with blood lead concentrations < 10 μ g/dL (Khalil-Manesh *et al.*, 1994; Ding *et al.*, 1998; Vaziri *et al.*, 1999b). BP effects at these exposure levels have been observed in two different rat strains, but all of these studies were exclusively on male animals. None of the recent, low dose studies reported an idenfiable threshold for the effects on BP.

The studies conducted by Attri *et al.* (2003) and Ding *et al.* (1998) are presented in more detail as examples of this extensive literature.

In the study by Attri *et al.* (2003), adult male Wistar-Kyoto rats (age not reported; weight 150-200g) were divided into control and lead-exposed groups of 10 animals⁸ each. lead-exposed animals were administered 1 ml 0.01% lead acetate (100 ppm) in water orally by syringe daily⁹ for up to 3 months. Blood pressure was measured

⁸ A third treatment group was exposed to Pb and vitamin C, but these results are not discussed here

⁹ Equivalent to a dose of 0. to .7 mg/kg/d.

monthly by tail-cuff; animals were rested 20 minutes prior to measurement in a restrainer cage, animals were conditioned to the procedure, and the measurement was recorded as the mean of 3 consecutive readings. Blood lead levels were significantly (p<0.05) elevated in the lead-exposed animals after 2 months of exposure and were 2.4 ± 0.6 (SEM) µg/dL. The lead-exposed animals also had statistically significant (p<0.05) higher systolic blood pressure; decreased plasma nitrite; decreased endogenous antioxidants as measured by plasma Vitamin A (retinol) and Vitamin E (α -tocopherol), decreased ferric-reducing antioxidant power (FRAP) – a measure of total antioxidant status; increased lipid peroxidation as measured by malondialdehyde (MDA), and increased oxidative DNA damage as measured by agarose gel electrophoresis and 8-hyroxy-deoxyguanosine (8-OH-dG).

Similar results were reported by Ding et al. (1998), where a blood lead of approximately 3 µg/dL in rats resulted in an elevation in mean BP of about 30%. Seven two-month old male Sprague-Dawley (SD) rats (weighing 200g) were exposed to 0.01% lead acetate (100 ppm lead) in drinking water for 12 weeks. An equal number of age-matched animals were used as controls. Animals were observed for an additional two weeks after exposure was stopped. BP was measured during lead exposure with a tail cuff: animals were rested 15 minutes prior to measurement in a restrainer cage, animals were conditioned to the procedure, and the measurement was recorded as the mean of 4 consecutive readings. After the 12 week lead exposure period, BP was measured in thiobutabarbital anesthetised animals via catheter in the left carotid artery. The blood lead concentration in lead exposed rats at 14 weeks was 3.2 ±0.2 (SEM) µg/dL and significantly (p < 0.05) greater than that of the control group (<1.0 μ g/dL). Mean BP in the lead exposed rats was significantly greater than that of the control group at eight weeks of exposure and continued to rise until lead exposure was stopped at week 12. Mean BP in the lead exposed rats remained elevated after cessation of lead exposure at 12 weeks and was 149 ±2 mmHg relative to 116 ±2 mmHg in controls at 14 weeks. The results of this experiment are illustrated in Figure 10 below. Also shown in Figure 10, is the ability of the oral chelator 2,3-dimercaptosuccinic acid (DMSA) to reduce the lead-induced hypertension. These results are not discussed here.

Boscolo and Carmignani (1988) reported a non-significant increase in BP of SD rats exposed to 15 ppm lead acetate in drinking water for 18 months (n=10). The blood lead concentration of the rats in this exposure group at 18 month was 7.4 \pm 0.5 (SEM) µg/dL. This was not considered as a defensible NOAEL because the blood lead concentration in the control group was 3.9 \pm 0.2 µg/dL, within the effects range reported by other, more recent studies (Ding *et al.*, 1998; Attri *et al.*, 2003).

Malvezzi *et al* (2001) reported femur lead concentrations in association with elevated BP in rats, but the exposure levels (700 ppm lead acetate) were much higher than the above studies.

Consistency of Evidence

Lead exposure has been reported to result in increased BP in three month old dogs (Fine *et al.*, 1988) and male pigeons (Revis *et al.*, 1981) at exposures that are at least as low as those where an effect has been demonstrated in male rats.

The results of recent, relatively low dose chronic oral rat studies have consistently reported that lead exposure causes elevations in BP. However, the findings from older, relatively higher dose studies are not as consisten:t As an example, Lal *et al.* (1991) exposed rats for 90 days to 0.25, 0.5 and 1% (equivalent to 2,500; 5,000; and 10,000 ppm) lead (as lead acetate) in drinking water and reported 0.25 as a NOAEL for increased arterial BP and calcium influx in cardiac muscle tissue (atrial trabeculae and papillary muscles). However, other studies (Khalil-Manesh *et al.*,

1994; Ding *et al.*, 1998; Vaziri *et al.*, 1999a; Vaziri *et al.*, 1999b) have reported a significant BP response at doses that are 25 fold lower than the NOAEL reported by Lal *et al.* (1991) of 0.01% (100 ppm).

Some have suggested that this body of data constitutes evidence of a biphasic dose-response, with greater sensitivity or response seen at relatively low doses (Victery, 1988; IARC, 2006). For example; several studies have reported that chronic oral exposure of rats to 100 ppm lead in drinking water resulted in increased BP, whereas no response was elicited at 500 ppm lead (Victery et al., 1982; Khalil-Manesh et al., 1993b; Khalil-Manesh et al., 1994). A recent chronic study suggests that the BP effects of lead exposure may not only be dose dependent, but may also depend on the relative timing of lead exposure and BP measurement. Bagchi et al. (2005) reported that young rats exposed to relatively high oral doses of lead (1%) lead acetate in drinking water) for 40 days had a transient increase in SBP relative to controls over the first 10 days of exposure. The SBP of lead exposed rats was then observed to fall back to control levels for the duration of the exposure and following exposure until approximately 120 days after the start of the experiment. At 120 days, the SBP of the lead exposed rats continued to increase with age, whereas the SBP of control and Na₂CaEDTA chelated rats showed a steady decline with advancing age. The blood lead of exposed rats exceeded 240 µg/dL at day 22 of this experiment (half way through the lead exposure period), yet the BP of the exposed rats had returned to control values by this point. This suggests that the net effect of very high lead exposures on BP may be neutral, whereas a positive effect is found at relatively low exposures (as would have been experienced over the early days of lead exposure in this study, where a transient rise in BP relative to controls was reported.

Collectively these data suggest that the dose-response relationship for lead and BP is complex – it may be non-linear, biphasic and dependent not only on the

magnitude, duration, and timing of exposure, but also on the timing of BP measurement relative to exposure. These factors would contribute to the difficulty of elucidating a dose-response relationship for this endpoint in epidemiological research, where the investigators ability to measure and account for these variables is limited. While the precise nature of the dose-response relationship between lead exposure and BP has yet to be described and a mechanism for a biphasic doseresponse has not been adequately established, the evidence from animal experiments has consistently demonstrated a significant positive association between low dose chronic and subchronic lead exposure and BP.



Time (weeks)

Figure 10. (Reproduced from Ding et al. (1998) Time course of mean (± SEM) blood pressure after administration of lead acetate (100 ppm) and the chelator 2,3-dimercaptosuccinic acid (DMSA) (0.5% in drinking water) to male rats. ##P<0.01 (ANOVA), *P<0.05, *P<0.01 compared to week 12.

4.4 MECHANISMS OF VASCULAR TOXICITY OF LEAD

The collective evidence from epidemiological studies and *in vivo* and *in vitro* experimental studies clearly indicate that there several mechanisms by which chronic lead exposure could cause elevated blood pressure and related cardiovascular disease. The literature contains evidence that lead depresses NO and impairs NO signalling, induces vascular inflammation, increases adrenergic sensitivity, increases endothelins, interferes with the functioning of the renninangiotension-aldosterone and kininergic systems, upsets the balance of vasodilator and vasoconstrictor prostaglandins, induces calcium dependent and calcium independent contractility of vascular smooth muscle cells, stimulates proliferation and phenotypic transformation of vascular smooth muscle cells, inhibits proliferation of endothelial cells and impairs angiogenesis and endothelial cell repair, and inhibits sodium-potassium adenosine triphosphatase. Much of the evidence of these effects is derived from studies of chronic, environmentally relevant lead exposures. Lead exposure may also induce hypertension as a secondary effect of cardiotoxicity or nephrotoxicity. However, it is important to note that the hypertensive effects of lead have been repeatedly demonstrated to be independent of adverse cardiac or nephrotoxic effects.

Lead-induced hypertension may result from increased cardiac inotropism (increased stroke volume and cardiac output) and increased peripheral resistance. There is evidence that lead can affect hypertension via both of these pathways but the direct effects on peripheral resistance appear more sensitive and pronounced and the emphasis here will be on this pathway.

Oxidative Stress

Chronic low level lead exposure has been shown to inactivate nitric oxide (NO) via production of reactive oxygen species (ROS). NO regulates blood pressure by promoting vasodilation (activates sGC to produce cGMP), renal excretion of Na²⁺ and water, and inhibits the sympathetic nervous system. The importance of NO in regulating vascular tension is demonstrated by the severe hypertension induced by NO inhibitors. Oxidative stress can also promote activation of the nuclear transcription factor kappa B (NF κ B) which triggers inflammation and apoptosis.

Lead exposure produces reactive oxygen species (ROS), such as superoxide ($O2\cong$), hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH•) via a Fenton reaction (Vaziri *et al.*, 2001; Ni *et al.*, 2004). Vaziri *et al.* (2001) demonstrated that treatment of lead-hypertensive rats with the cell-permeable SOD-mimetic drug tempol reversed NO mediated hypertension. These results suggest that oxidative stress and elevated OH• generation observed in lead exposed rats is primarily due to increased O2•-, which is a precursor to OH•. Superoxide can oxidize NO, thereby inactivating NO and producing the consequent vascular effects. The product of the superoxide and NO is the oxidant (and nitrating agent) peroxynitrite (ONOO-). Peroxynitrite is highly reactive with a variety of cellular components, including DNA and proteins, and may contribute to other (non-vascular) adverse effects such as cadiac, renal and neurological toxicity (US EPA, 2006).

Evidence of oxidative stress is present in humans or animals with hypertension, independent of the cause of hypertension. Similarly, antioxidants or dietary modifications in humans or animals with hypertension reduces oxidative stress, increases available NO and reduces BP. Finally, induction of oxidative stress by glutathione depletion results in antioxidant treatable hypertension (Vaziri *et al.*, 2001).

Chronic exposure of rats to100 ppm lead in drinking water produces hypertension and:

- Increased plasma and tissue concentrations of malondialdehyde (MDA), a marker of lipid peroxidation and oxidative damage (Gonick *et al.*, 1997; Attri *et al.*, 2003)
- Reduced plasma levels of available NO and increased urinary excretion of NO metabolites, NOx (Vaziri *et al.*, 1997; Vaziri *et al.*, 1999b; Dursun *et al.*, 2005)
- Increased plasma and brain, renal and cardiovascular tissue concentrations of nitrotyrosine, a marker of oxidation of NO by ROS (Vaziri *et al.*, 1999b)
- Compensatory up-regulation of renal, cardiac, and vascular endothelial (eNOS) and inducible (iNOS) NO sythase expression (Gonick *et al.*, 1997; Vaziri *et al.*, 1997; Vaziri *et al.*, 1999a; Vaziri *et al.*, 2001)

Treatment of lead-hypertensive rats with antioxidants, such as vitamin E, vitamin C, lazaroid (16 desmethyl-tirilazad), and tempol has repeatedly been reported to reduce BP, increase NO availability, reduce oxidative damage, and reverse compensatory upregulation of nitric oxide synthase (NOS) istotypes independent of any effect on blood lead concentrations. Cessation of antioxidant treatment has been reported to reduce such effects (Khalil-Manesh *et al.*, 1994; Vaziri *et al.*, 1997; Vaziri *et al.*, 1999a; Ding *et al.*, 2001; Vaziri *et al.*, 2001; Attri *et al.*, 2003)

Ding *et al.* (1998) also reported that infusion with NOS substrate L-Arginine is more effective at mitigating lead-induced hypertension than oral DMSA, providing further evidence that lead related deficits in NO contribute to lead's hypertensive effects.

NO Signalling

NO regulates vascular tension via the enzyme soluble guanylate cyclase (sGC) and the cellular messenger cyclic guanosine monophosphate (cGMP). NO activates sGC to produce cGMP; cGMP lowers cytosolic Ca 2+ and thereby promotes vasodilation. *In vivo* and *in vitro* studies demonstrate that lead exposure can downregulate vascular tissue expression of sGC via the combined effects of oxidative stress and upregulation of cyclooxygenase-2 (COX-2) (Courtois *et al.*, 2003; Farmand *et al.*, 2005). Lead exposure also lowered plasma and urinary cGMP in rats (Khalil-Manesh *et al.*, 1993b).

Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cells (NF-KB)

NF-κB is sensitive to oxidative stress and chronic exposure to lead in drinking water has been shown to activate NF-κB in rat brain and renal tissues (Ramesh *et al.*, 2001; Rodriguez-Iturbe *et al.*, 2005). The activation of NF-κB can lead to inflammation and apoptosis. Inflammation contributes to the pathogenesis of hypertension. An inflammatory response has been observed in renal and vascular tissues of lead-hypertensive rats (Carmignani *et al.*, 2000; Rodriguez-Iturbe *et al.*, 2005).

Adrenergic System

The adrenergic system, or sympathetic nervous system, regulates blood pressure, cardiac function and haemodynamics. There is significant evidence that lead directly stimulates or, via its previously discussed ROS deactivation of NO, inhibits the suppression of the adrenergic system. Stimulation (or inhibition of supression) of the adrenergic system results in the "fight or flight" response, including increased BP. Chronic lead exposure has been associated with increased plasma norepinephrine, reduced vascular ß-andrenergic receptor density, decreased cAMP

production, and reduced vasoconstrictor response to ß-andrenergic stimulation in the vascular tissue.

Occupationally lead exposed humans exhibited elevated plasma norepinephrine (NE), but not epinephrine (E) or dopamine (DA) (Chang *et al.*, 1996). Similarly, (Chang *et al.*, 1997; Tsao *et al.*, 2000) reported elevated NE, but not E in subchronically lead-exposed rats. This effect was accompanied by a reduction in the density of ß-andrenergic receptors and reduced cyclic-3',5'-adenosine monophosphate (cAMP) production in vascular and cardiac tissues and an increase in the density of ß-andrenergic receptors and increased cAMP production in renal tissue. These effects were partially reversed in a stop exposure study (Chang *et al.*, 2005). Carmignani *et al.* (2000) also reported increased plasma catecholamines and cardiac contractility in lead-exposed rats. Reduce cardiac and vascular ß-andrenergic receptor density can result in increased vascular resistance. Increased renal ß-andrenergic receptor density can result in the release of renin.

Protein Kinase C (PKC)

Protein kinase C (PKC) are cellular messengers that are involved in the induction of adrenergic response of the cardiovascular system, including regulation of vascular tone and blood flow. Occupational exposures and *in vitro* studies at μ M levels have reported associations between lead exposure and increased PKC activity. An additional *in vitro* study demonstrated that PKC mediated the contractility of artery cells exposed to sub nM concentrations of lead¹⁰ independent of the influence of endothelium or the extracellular influx of Ca²⁺ (Watts *et al.*, 1995). There is evidence that this mechanism of action (PKC) may be tissue and concentration specific – lead

 $^{^{10}}$ 1 nM Pb, assuming a plasma:RBC binding of 0.24%, is approximately equivalent to 9 μg Pb/dL whole blood.

inhibits PKC at higher concentrations (Watts *et al.*, 1995; Valencia *et al.*, 2001; Lidsky and Schneider, 2003).

Renin-Angiotensin-Aldosterone (RAAS) & Kininergic Systems

The renin-angiotensin-aldosterone (RAAS) & kininergic systems are paracrine systems that regulate blood pressure through a series of complex feedback mechanisms. There is evidence that lead exposure can induce the RAAS (increase plasma rennin), but the responses are variable and appear to be dependent on dose, duration of exposure, and age at exposure. Acute and subchronic lead exposure appears to result in an increase in plasma renin, whereas plasma renin levels may return to normal after cessation of the chronic lead exposure. The observed upregulation of renal ß-andrenergic receptor density may result in increased renin release. There is also evidence that chronic, environmentally relevant lead exposure in rats increases plasma angiotensin I-converting enzyme (ACE), kininase II, and aldosterone (Boscolo and Carmignani, 1988; Carmignani et al., 1999). A more recent chronic rat study by Bagchi et al. (2005) indicates that induction of the RAAS may be partly responsible for chronically elevated BP in lead treated rats. These authors reported that administration of the angiotensin II receptor blocker Losartan reduced BP in lead-hypertensive rats both during a 40 day exposure period and up to 282 days post exposure. Additional evidence, including increased water intake, urinary output, and urinary potassium and decreased urinary sodium among lead exposed rats is consistent with an aldosterone effect secondary to angiotensin II stimulation.

Prostaglandins

Prostaglandins are widely distributed autocrine and paracrine mediators. Thromoxan (TXB₂) is a vasoconstricive prostaglandin and 6-ketoPGF1 is a vasodilatory
prostaglandin. Evidence form occupational studies (Cardenas *et al.*, 1993; Hotter *et al.*, 1995) and *in vitro* experiments (Dorman and Freeman, 2002) suggests that lead exposure can upset the balance of the above-noted vasodilator and vasoconstrictor prostaglandins. Similar effects were not observed in a rat study (Gonick *et al.*, 1997), however.

Endothelins

Several rat studies have reported that an increase in plasma endothelin (ET) levels or increased urinary excretion of ET is associated with lead-induced hypertension (Khalil-Manesh *et al.*, 1993b; Khalil-Manesh *et al.*, 1994; Gonick *et al.*, 1997). Additional evidence suggests that increased ET may be responsible for depressing sCG expression and cGMP production (Courtois *et al.*, 2003).

Endothelian and Vascular Smooth Muscle Cells

Endothelial damage or dysfunction can lead to hypertension, atherosclerosis, thrombosis and tissue injury. Chronic lead exposure has been demonstrated to result in atherosclerosis in male pigeons (Revis *et al.*, 1981). *In vitro* studies reported that lead exposure (0.5μ M- 100μ M) inhibits proliferation of endothelial cells and impairs angiogenesis and endothelial cell repair (Kaji *et al.*, 1995a; Kaji *et al.*, 1995b; Ueda *et al.*, 1997; Fujiwara *et al.*, 1998). *In vitro* studies have also shown that lead can promote vascular smooth muscle cell and fibroblast proliferation and phenotypic transformation (Carsia *et al.*, 1995; Fujiwara *et al.*, 1995; Fujiwara and Kaji, 1999). An *in vitro* experiment demonstrated that lead (0.1 to 3.1 mM) could induce vasoconstriction of vascular smooth muscle cell independent of Ca²⁺ (Valencia *et al.*, 2001). Lead may also induce vasoconstriction via Ca²⁺ dependent pathways, such as activation of PKC. Collectively, these effects could contribute to increased blood pressure, atherosclerosis, thrombosis and tissue injury.

Gump *et al.* (2005) reported a positive association between early childhood blood lead and increased total peripheral resistance (TPH) in response to acute stress in 9.5 year old children. There is evidence that increased TPR leads to higher blood pressure and ventricular hypertrophy, which, aside from age, is the strongest known predictor of cardiovascular morbidity and mortality. Increased TPR can lead to hypertension either directly, through structural remodeling or resistance vessels or thickening of vascular walls, or indirectly via the development of atherosclerosis.

Sodium-potassium Adenosine Triphosphatase

Sodium-potassium adenosine triphosphatase (Na⁺-K⁺ATPase) catalyzes the active transport of Na⁺ and K⁺ across the cell membrane. Lead inhibits Na⁺-K⁺ATPase *in vitro* and reductions in erythrocyte membrane Na⁺-K⁺ATPase have been associated with occupational and environmental lead exposures. In separate studies, reduced erythrocyte membrane Na⁺-K⁺ATPase has been associated with risk of hypertension and increased BP. Glenn *et al.* (2001) reported that a restriction fragment length polymorphism (RFLP) at the 3' end of the Na⁺-K⁺ATPase 2 α gene significantly modified the association between blood and tibia lead and SBP of a cohort of male former organolead workers which suggests that the expression or the functioning of the Na⁺-K⁺ATPase enzyme is important in the aetiology of lead-induced hypertension in humans.

4.5 VARIABILITY IN SUSCEPTIBILITY TO THE VASCULAR TOXICITY OF LEAD

The objective of the following section is to review the evidence of potential variations in susceptibility to the effects of lead exposure on BP.

There is considerable variability in the reported biomarker-response relationships for both blood lead and bone lead and SBP. While there is suggestive evidence of the influence of some of the variables discussed below, it is not possible to isolate and quantify the influence of any single variable.

Age

As reviewed above, the limited existing epidemiological literature does not support an association between blood lead and SBP in children. The existing evidence does not indicate that children are any more susceptible to the hypertensive effects of lead than adults.

Sex

Animal evidence also suggestive of differences between the sexes - with male rats more susceptible to hypertensive effects (IARC, 2006). However, the presence of a difference is difficult to isolate because studies differ in strain, age of exposure, duration of exposure, route of exposure, lead compound and method, timing and frequency of BP measurement. At least one study (Victery *et al.*, 1982) reported that male rats were more sensitive to the hypertensive effects of pre- and postnatal lead exposure than females under similar experimental conditions. No mechanistic explanation has been offered for the reported difference between sexes. Therefore an evaluation of whether this mechanism would also be expected to produce a similar difference in humans cannot be currently conducted.

There have been inconsistent findings of sex related difference in blood lead-SBP relationships among epidemiological studies. For example, no significant sex effect was evident in the meta-analysis by Nawrot *et al* (2002), whereas Vupputuri *et al*.

(2003) reported that the relationship between blood lead and SBP differed significantly between the sexes.

Ethnicity

Some, but not all, epidemiological studies report a greater blood lead effect on SBP for African-Americans than Caucasians (Vupputuri *et al.*, 2003; Den Hond *et al.*, 2002). Again, there is insufficient evidence to conclude that ethnicity in isolation is a significant contributing factor to the observed variation in biomarker-response relationships for blood and bone lead and SBP.

Nutritional Status

It is hypothesized that nutritional status will influence the biomarker-response relationship for blood and bone lead and SBP, but the overall direction of effect is difficult to predict. There is evidence that calcium deficiency may increase the susceptibility to BP effects of lead, but calcium is only one of many potentially influential nutritional variables and the evidence of its effect is limited. Therefore the existing evidence, summarized below, is insufficient to develop a quantitative biomarker-response relationship for calcuim deficient subpopulations.

Bogden *et al.* (1995) reported that SD rats exposed to 250 ppm lead (lead acetate) in drinking water and a low calcium diet ($0.1\% \text{ Ca}^{2+}$ as CaCO₃) had significantly increased SBP at the 3rd trimester of pregnancy. Dams fed 0.5 and 2.5% Ca²⁺ at this level of lead exposure had a non-significant increase in SBP at the 3rd trimester of pregnancy. This study demonstrates that calcium deficiency may increase the susceptibility of pregnant rats to the vascular effects of lead. However, Revis *et al.* (1981) reported that Ca²⁺ was ineffective at reducing the hypertensive effect of lead in male pigeons.

Elmarsafawy *et al.* (2006) reported that calcium modified the relationship between tibia lead and blood lead and hypertension among 471 subjects of the Normative Aging Study (NAS). These findings support an earlier report by Pizent *et al.* (2001) that low calcium intake can modify the relationship between blood lead and DBP among 267 non-smoking, middle-aged to elderly women from rural Croatia.

Stress

Psychological stress may intensify the vascular effects of chronic lead exposure. After controlling for the confounding influence of demographic and behavioural risk factors, the dose-response relationship between bone lead (tibia and patella) and risk of hypertension was approximately 2½ fold higher among subjects of the NAS who self-reported high stress (Peters *et al.*, 2007). The respective dose-response relationships between tibia lead and SBP for high and low stress subjects from this study are illustrated in Figure 12 below. While this important study is indicative of the large influence that a potentially modifying effect like stress can have on the biomarker-response relationship, the findings have yet to be replicated.

Co-Exposure to Other Xenobiotics

Co-exposure to other environmental chemicals and drugs may modify the biomarker-responses between blood lead and bone lead and SBP. Kaji *et al.* (1995b), for example, has shown that lead enhanced the *in vitro* cytotoxicity of cadmium to bovine aortic endothelial cells. The overall population effect, however, of the myriad potential interactive effects is impossible to predict.

Genetic Variability

The modifying effect of several single nucleotide polymorphisms (SNPs) on blood and bone lead relations with BP or hypertension has been investigated. These SNPs include endothelial nitric oxide synthase (eNOS), vitamin D receptor (VDR), δ aminolevulinic acid dehydratase (ALAD), and Sodium-potassium Adenosine Triphosphatase $\alpha 2$ (ATP1A2). Of these, ALAD has received the most attention with respect to its potential modifying influence on the toxicokinetics and toxicodynamics of lead. However, ATP1A2 is the only SNP with evidence to suggest that it has the ability to modify the blood lead or bone lead and SBP biomarker-response relationship. One study reported that subjects that were homozygous for a variant allele of this gene had a tibia lead-SBP biomarker-response relationship that was over 10-fold stronger than those that were heterozygous or homozygous for the wildtype allele (Glenn *et al.*, 2001). While these findings are suggestive of the existence of a very sensitive subpopulation, additional investigations are needed to corroborate these results.

eNOS

Lustberg *et al.* (2004) evaluated whether the G -T polymorphism in exon 7 of the endothelial nitric oxide synthase (eNOS) gene is associated with blood pressure or modifies the relation between lead dose and blood pressure in 803 lead workers in Korea. Lustberg *et al.* reported that there was no evidence of effect modification by eNOS genotype on relations of lead dose with blood pressure. This finding is particularly interesting given the well established mechanism of lead-induced hypertension involving endothelial NO.

VDR

*Bsm*l polymorphisms of the vitamin D receptor (VDR) gene can affect both lead toxicokinetics and BP. Korean lead workers with the VDR *B* allele had significantly

higher blood lead, chelatable lead, and tibia lead than co-workers with the VDR *bb* genotype (Schwartz *et al.*, 2000a). Schwartz *et al.* (2000a) showed the VDR genotype is independently associated with BP and hypertension, with subjects with the *B* allele demonstrating higher SBP and DBP and greater risk of hypertension after adjustment for covariates.

VDR genotype (BB and Bb *vs.*, bb) were positive predictors of BP. Subjects with the VDR B allele (11.2%) had higher average SBP and DBP, larger increases in BP with age, and higher prevalence of hypertension than those subjects with the bb genotype. However, VDR genotype did not modify association of blood lead, tibia lead or (DMSA)-chelatable lead with SBP (Lee *et al.*, 2001).

ALAD

Lee *et al.* (2001) reported that δ -aminolevulinic acid dehydratase (ALAD) genotype had no observable independent effect on BP nor modified the relationship between lead and BP.

Sodium-potassium Adenosine Triphosphatase a2 Gene

Glenn *et al.* (2001) investigated the modifying of 2 restriction fragment length polymorphisms (RFLPs) in the Na⁺-K⁺ATPase α 2 (ATP1A2) gene on the relationships between blood and bone lead and BP and hypertension among 226 middle aged, predominantly Caucasian males formerly occupationally lead exposed at a New Jersey chemical manufacturing facility (this study cohort is described in more detail above). Subjects were genotyped according to restriction fragment length at the 5' end (8.0/8.0, 8.0/3.3, and 3.3/3.3 kilobases) and at the 3' end (10.5/10.5, 10.5/4.3, and 4.3/4.2 kilobases) of the ATP1A2 gene. Multiple linear regression and logistical regression were used in cross-sectional analysis of the

relationships between ATP1A2 RFLPs, lead exposure, relevant covariates and BP and hypertension, respectively. The ATP1A2 (3') RFLP significantly (p = 0.01) modified the association between blood lead and tibia lead and SBP. The covariate adjusted linear regression coefficient for the subjects homozygous for the 10.5 allele was approximately 12 fold higher (6.1 mmHg per µg/dL) than that for those with the 10.5/4.3 or 4.3/4.3 genotype (0.5 mmHg per µg/dL). The modifying effect of the ATP1A2 (3') RFLP on the dose-response relationship for blood lead and SBP is illustrated in Figure 11. The authors report that the ATP1A2 (3') RFLP also had a significant modifying effect on the relationship between tibia lead and SBP; however, the data and results were not presented for this biomarker of lead exposure. ATP1A2 (3') did not significantly modify the relationship between blood lead or tibia lead and BP and no associations were found between blood or tibia lead and BP and the RFLP of ATP1A2 (5'). Neither the 3' nor the 5' RFLP of ATP1A2 modified the relationship between blood or tibia lead and hypertension.

The prevalence of the 10.5 kilobase ATP1A2 (3') allele was 25% among this study cohort, with 5% of subjects homozygous. It is not known whether these results have been replicated; however, they are suggestive that up to 5% of the male Caucasian population could be very sensitive to the effects of lead on SBP with a dose-response relationship that is an order of magnitude steeper than that observed in the balance of the population. The heterogeneity of responses observed in this study cohort also suggest that epidemiological studies of this relationship that do not control for the modifying effect of this RFLP risk under-estimating the association between lead exposure and SBP. The10.5 kilobase ATP1A2 (3') allele is reportedly more prevalent among African-Americans, which may partly explain the apparent sensitivity of African-Americans reported by some researchers.



Figure 11. Reproduced from Glenn et al. (2001). Adjusted linear regression models of association between blood lead and systolic blood pressure among 220 former New Jersey organolead workers. Subjects were stratified by genotype for a restriction fragment length polymorphism at the 3' end of the sodium-potassium adenosine triphosphatase α2 subunit gene. The symbols represent subject genotype: circle = 4.3/4.3 or 4.3/10.5; triangle = 10.5/10.5.



Figure 12. From Peters et al. (2007). The adjusted dose-response relationships between tibia lead and systolic blood pressure (SBP) for participants of the Normative Aging Study whose self reported stress was either high (dashed line) or low (solid line).

Summary

There are several variables that appear to be able to confer susceptibility to the effects of lead on SBP and potentially modify the blood lead and tibia lead biomarker-response relationships for this endpoint. These variables include stress, variation in the ATP1A2 gene, and calcium deficiency. These variables may increase the slope of the relationship between blood lead and SBP by up to a factor of 10.

4.6 BIOMARKERS AND CARDIOVASCULAR ENDPOINTS RECOMMENDED FOR TRVS

As reviewed in Section 2, there is at least moderately strong evidence that blood lead concentrations less than 10 μ g/dL have adverse effects on the central nervous system, the immune system, the reproductive system, the cardiovascular system, haematopoiesis, and renal functioning. No threshold has been identified for any of these effects and one cannot identify a critical effect, as traditionally defined. Therefore, the limiting factor for TRV development for lead is the certainty with which a blood lead concentration-response relationship can be quantified.

Within the domain of cardiovascular toxicity there are a number of biomarkerresponse relationships that have been quantified in the literature and could potentially form the basis for TRV development. These include adult blood lead and SBP, DBP, or risk of hypertension; adult bone lead and SBP, DBP, or risk of hypertension; children's blood lead and SBP, DBP, or total peripheral vascular resistance; and maternal blood lead and SBP in children. However, the endpoint for which the biomarker concentration-response has been most often investigated and which can be characterized with the greatest certainty is the relationship between adult blood lead concentration and increased SBP.

Very few of the available studies report a significant positive association between blood lead and SBP or DBP in children: Only one of five studies published before the 1990's on the relationship between children's blood lead and blood pressure report a significant positive association. Two of the three recent prospective epidemiological studies (Factor-Litvak *et al.*, 1996; Chen *et al.*, 2006) report no significant association between children's blood lead and blood pressure, while the results from the Oswego Children's Study (Gump *et al.*, 2005) suggest that early life lead exposure can result in cardiovascular effects that may initially have little net effect on BP, but which may ultimately lead to hypertension or other cardiovascular morbidity and mortality. This evidence, however, has not yet been replicated. Considered collectively, the currently available epidemiological data does not provide a sufficiently certain estimate of the relationship between children's blood lead or maternal blood lead and SBP or other cardiovascular endpoints in children.

From the current understanding of lead toxicokinetics, bone lead is expected to provide a better measure of cumulative lead exposure than blood lead and, therefore, bone lead would have a stronger relationship to chronic health endpoints than blood lead measurements. No published studies on the relationship between bone lead and BP in children were identified. Tibia lead has been the most commonly measured bone lead metric in epidemiological studies of cardiovascular effects in adults. Associations of tibia lead and SBP (as well as SBP and risk of hypertension) have not been consistent in the available epidemiological studies. The relationship between tibia lead and SBP has been examined in a total of seven cohorts - three occupational and four environmental. A significant positive association, after adjusting for covariates, was reported in three of the seven cohorts. Three of the six reported no significant association, and a significant negative association was observed in the longitudinal analysis of the South Korean lead workers. The absence of reporting for this endpoint in some of the studies raises suspicions of publication bias. No clear trend is evident with respect to results from occupational or environmental cohorts. Considered collectively, the currently available epidemiological data does not provide a sufficiently certain estimate of the relationship between adult tibia lead and SBP or other cardiovascular endpoints in children.

An additional issue that must be considered when weighing whether to derive a TRV on the basis of both bone lead and blood lead for the same endpoint (SBP) is that the two TRVs must be internally consistent. Uncertainty in the longitudinal relationship between bone lead and blood lead is sufficiently large that it precludes the ability to provide a meaningful check whether derived exposure-response

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relationships for bone lead are compatible with the exposure-response relationships derived for blood lead.

In summary, the biomarker-endpoint relationship that can be characterized with the greatest certainty for the cardiovascular toxicity of lead is the relationship between adult blood lead concentrations and SBP. While bone lead shows promise as a complimentary biomarker for the effects of cumulative lead exposure, the bone lead concentration–response relationships have not been nearly as well characterized as the blood lead concentration response relationships and are currently not sufficiently certain to derive a meaningful TRV.

4.7 SUMMARY AND CONCLUSIONS

This section of the report:

Provided a review of the available evidence from epidemiological studies on the association between chronic lead and increased blood pressure. The epidemiological evidence of an association between blood lead and elevated BP in adults is inconsistent, but suggestive. Several meta-analyses report a weak, significantly positive association and three of six longitudinal studies published since 1980 report a significant association between blood lead and SBP.

The epidemiological evidence of an association between tibia lead and elevated BP is also inconsistent, but suggestive. A significant positive association, after adjusting for covariates, has been reported between tibia lead and SBP in three of the seven cohorts where this relationship has been studied.

The epidemiological evidence of an association between lead exposure and elevated BP in children is mostly negative and is currently insufficient to derive a

toxicological reference value for this endpoint and lifestage. One good quality study reports an association between lead exposure in children and adverse effects on cardiovascular functioning that could lead to latter life development of hypertension and other cardiovascular morbidity and mortality.

Reviewed the available evidence from in vivo experiments on the association between chronic lead exposure in laboratory animals and increased blood

pressure. Both chronic and subchronic oral exposure to lead in laboratory animals results in elevated blood pressure. This effect has been demonstrated in multiple species and has been repeatedly demonstrated in multiple rat strains. The result of more recent animal studies have consistently shown an effect at relatively low levels of lead exposure – statistically significant increases in the blood pressure of rats have been reported at blood lead concentrations as low as 2.4 μ g/dL.

Reviewed the available evidence on plausible mechanisms of action by which chronic lead exposure could result in increased blood pressure. The collective evidence from epidemiological studies and *in vivo* and *in vitro* experimental studies clearly indicate that there several mechanisms by which chronic lead exposure could cause elevated blood pressure and related cardiovascular disease. The mode of action for which there is the most evidence is vasoconstriction secondary to leadinduced oxidative stress and subsequent inactivation of the vasodilator nitric oxide (NO) and related signalling pathways and functional responses.

Reviewed the available evidence on variability in response. There are several variables that appear to be able to confer susceptibility to the effects of lead on SBP and potentially modify the blood lead and tibia lead biomarker-response relationships for this endpoint. These variables include stress, variation in the ATP1A2 gene, and calcium deficiency. These variables may increase the slope of the relationship between blood lead and SBP by up to a factor of 10.

Justified, on the basis of the most certain biomarker-response relationship, the identification of candidate biomarker-endpoint combinations for TRV development. The endpoint for which the blood lead concentration-response has been most often investigated and which can be characterized with the greatest certainty is the relationship between adult blood lead concentration and systolic blood pressure (SBP). The biomarker-response relationships for other biomarkers, such as bone lead, maternal lead, children's blood lead and other cardiovascular endpoints, such as diastolic blood pressure (DBP) and risk of hypertension are relatively more uncertain. Therefore,TRVs were not derived explicitly for these alternate biomarker-endpoint relationships.

SECTION 5 • ESTIMATED BLOOD LEAD CONCENTRATION-RESPONSE SLOPES

5.1 INTRODUCTION

The objective of this section is to recommend quantitative estimates of the blood lead concentration-response relationships for IQ in children and systolic blood pressure (SBP) in adults, with an emphasis on quantifying blood lead concentration-response relationships over the range of current blood lead concentrations in Canadians. Estimates of the blood lead concentration-response relationships are provided so that risk assessors can estimate the change in population health outcomes associated with incremental changes in environmental lead exposures. Estimates of the slopes of blood lead concentration-response relationships can be made with a relatively high degree of scientific certainty, as the available blood lead concentration-response data from epidemiological studies are within, or very close to within, the range of current blood lead concentrations in Canadians. In contrast, data from critical studies for cancer are from exposures that are about 1,000 times higher than current environmental lead exposure levels in Canada; therefore, no estimate of the exposure-response relationship for cancer is provided in this section (see Section 7 for cancer TRVs).

A secondary purpose for quantifying lead exposure-response¹¹ relationships is for deriving toxicological reference values (TRVs). Because a population threshold for the toxicological effects of lead has yet to be clearly identified, the TRVs for lead will necessarily be based on a policy decision on a "target" level of population health risk. Also, where the study data are derived from lead exposures levels that are

¹¹ The term exposure-response relationship includes blood lead concentration-response relationships.

much higher than contemporary environmental exposures, such as with cancer or where exposures associated with the target risk are below the range of study data (as is the case for IQ and SBP), the TRVs will be based on an extrapolated exposure-response relationship. The recommended TRVs are derived in other sections of the report due to the requirement for a policy choice on the target level of population health risk, as well as the requirement to extrapolate exposure-response relationships beyond the range of study data. These subjects are addressed in separate sections of the report in order to maintain a degree of separation between the estimates of exposure-response relationships, which are based on empirical evidence within the range of current environmental lead exposures in Canada, and the derived TRVs, which are based on extrapolated dose-response relationshipsand therefore are scientifically less certain- and are based on a policy decision about a target level of population health risk.

This section of the report:

Presents estimates of the current distribution of blood lead concentrations in environmentally exposed Canadians. Preliminary results from the nationally representative Canadian Health Measures Survey (CHMS) report that the 25th and 95th percentiles of blood lead concentrations among Canadians 20 to 79 years old in 2007 and 2008 were approximately 1.0 and 4.1 μ g/dL, respectively. The 25th and 95th percentiles of blood lead concentrations in children six to 19 years of age are approximately 0.6 and 2.1 μ g/dL, respectively. There are no nationally representative data available for Canadian children less than six years of age. By several lines of reasoning, the 25th and 95th percentiles of blood lead concentrations among Canadian children less than six years old are estimated to be 1 and 4-6 μ g/dL, respectively. Identifies a critical study that best represents the slope of the relationship between blood lead concentrations and IQ decrements in environmentally exposed Canadian children. An international pooled analysis of seven longitudinal studies by Lanphear *et al.* (2005) was selected as the critical study that best represents the expected blood lead concentration-IQ relationship among environmentally exposed Canadian children.

Critically reviews the sources of uncertainty and variability in the recommended slope of the blood lead concentration-IQ relationship. Most studies that have examined the shape and extent of the blood lead concentration-IQ relationship provide evidence that the slope of the relationship is steeper at lower blood lead concentrations. Multiple studies provide evidence that this relationship extends at least as low as 1 μ g/dL. While the weight of evidence supports this conclusion, the available studies are not unanimously conclusive, and there are other important sources of uncertainty and variability in the estimated slope of the blood lead concentration-IQ relationship. The 95th percent confidence intervals on the covariate adjusted regression coefficient from the critical study quantify some, but not all, of the uncertainty and variability in the overall estimate of the slope of the relationship.

Applies the recommended blood lead concentration-IQ slope to estimate the decrease in population mean IQ associated with an increase in children's blood lead concentrations from 1 to $4 \mu g/dL$ (estimated to be approximately the 25th and 95th percentile blood lead concentrations in Canadian children less than 6 years old). An increase in children's blood lead concentrations from 1 to $4 \mu g/dL$ is associated with an estimated decrement in population mean IQ of 2.3 to 5.2 IQ points, with a best estimate of 3.7 IQ points. The potential range of IQ decrements may be larger because the estimates provided do not account for all

potential sources of uncertainty and variability in the blood lead concentration-IQ relationship.

Identifies a critical study that best represents the expected slope of the relationship between blood lead concentrations and systolic blood pressure (SBP) in environmentally exposed Canadian adults. A longitudinal study of formerly occupationally exposed organolead workers by Glenn et al. (2003) was selected as the critical study that best represents the expected blood lead concentration-SBP relationship among environmentally exposed Caucasian males. However, because of the limited diversity of subjects in the study by Glenn et al. (2003), the variance in reported slopes of the blood lead concentration-SBP relationship, and the evidence of significant variance in susceptibility amongst subpopulations, a second critical study was identified to represent the potential blood lead concentration-SBP response among susceptible sub-populations. A crosssectional study of blood lead concentrations and SBP among adult female African-American subjects of the US National Health and Nutrition Examination Study by Vupputuri *et al.* (2003) was selected as the critical study that best represents the expected blood lead concentration-SBP relationship among susceptible subpopulations, including, but not limited to, African-American females.

Critically review the sources of uncertainty and variability in the recommended slope of the blood lead concentration-SBP relationship. Few analyses of the extent and shape of the blood lead concentration-SBP relationship are available in the literature. In the absence of compelling evidence to the contrary, it was concluded that the shape of the relationship was best represtented by a linear model. However, ecause the relationship has not been extensively tested for non-linearity, there is increasing uncertainty in the strength and extent of the relationship below blood lead concentrations of about 4 μ g/dL. There are other important sources of uncertainty and variability in the estimated slope of the blood lead concentration-

SBP relationship. The 95th percent confidence intervals on the covariate adjusted regression coefficients from the critical studies quantify some, but not all, of the uncertainty and variability in the overall estimate of the slope of the relationship.

Applies the recommended blood lead concentration-SBP slope to estimate the change in population mean SBP associated with an increase in adult blood lead concentrations from 1 to $4 \mu g/dL$ (approximately the 25^{th} and 95^{th} percentile blood lead concentrations in Canadian adults). An increase in adult blood lead concentrations from 1 to $4 \mu g/dL$ is associated with an estimated increase in population mean SBP of approximately 0.2 to 2.4 mmHg, with a best estimate of 0.8 mmHg among Caucasian males and 1.4 mmHg among susceptible sub-populations. However, the potential range of increase in SBP may be larger because the estimates provided do not account for all potential sources of uncertainty and variability in the blood lead concentration-SBP relationship.

Slopes of the blood lead concentration-response relationship for primary endpoints are provided so that risk assessors can calculate estimates in changes in health endpoints associated with changes in blood lead concentrations. However, it must be emphasized that:

- There is evidence that lead exposure can adversely affect many health endpoints, not just those for which quantitative estimates of the blood lead concentration-response relationship are provided in the report (IQ and SBP).
- The recommended blood lead concentration-response relationships describe the expected change in the <u>population mean</u> of the health endpoint associated with changing blood lead concentrations. Therfore, health effects at an individual level cannot be estimated from the blood lead concentrationresponse relationships recommended herein.
- In applying the recommended slopes, the uncertainty in the estimates of potential health effects associated with a change in blood lead concentration

should be acknowledged, presented transparently, and, where possible, quantified.

5.2 CURRENT BLOOD LEAD CONCENTRATIONS IN CANADIANS

The objective of this section is to provide estimates of the exposure-response relationships so that risk assessors can estimate the magnitude of change in population health risk associated with incremental changes in contemporary environmental lead exposures. This necessitates estimating the exposure-response relationships over the range of, and in close proximity to, contemporary Canadian lead exposures. The following discussion provides information on current distributions of blood lead concentrations in Canada. Estimates of current blood lead concentrations in Canada. Estimates of current blood lead concentrations from US national biomonitoring data because none are available for Canada.

Wong *et al.* (2008) provide preliminary estimates of national blood lead distributions for 2,678 subjects included in the Canadian Health Measures Survey (CHMS) conducted in 2007-08. These data was complied for Canadian children aged 6 to 19 and adults aged 20 to 79 – this study did not collect blood samples from children under the age of six. These data are preliminary because they are from only eight of a planned 15 collection sites for the CHMS. Nonetheless, they are the most representative data available of the current distribution of blood lead levels among Canadians 6 years of age and older. The 25th percentile, geometric mean, and 95th percentile blood lead concentrations (and 95% confidence intervals) for Canadian children 6 to 19 years old are 0.62 (0.55-0.69) μ g/dL, 0.88 (0.77-0.99) μ g/dL, and 2.05 (1.57-2.54) μ g/dL, respectively. The 25th percentile, geometric mean, and 95th percentile blood lead concentrations (and 95% confidence intervals) for Canadian adults 20 to 79 years old are 1.00 (0.88-1.12) μ g/dL, 1.50 (1.32-1.72) μ g/dL, and 4.11 (3.18-5.03) μ g/dL, respectively. These data are summarized in Table 15 below.

Age	Data Source	Reference	25 th Percentile	Geometric Mean	95 th Percentile
1-5 years	US NHANES III (2001-02)	US CDC, 2005	Not reported	1.70 (1.55-1.87)	5.80 (4.70-6.90)
6-19 years	CHMS 2007-08	Wong & Lye, 2008	0.62 (0.55-0.69)	0.88 (0.77-0.99)	2.05 (1.57-2.54)
20-79 years	CHMS 2007-08	Wong & Lye, 2008	1.00 (0.88-1.12)	1.50 (1.32-1.72)	4.11 (3.18-5.03)
≥20 years	US NHANES III (2001-02)	US CDC, 2005	Not reported	1.56 (1.49-1.62)	4.60 (4.20-4.90)

 Table 15. Blood lead concentrations as reported by Canadian and US national population biomonitoring

 studies. No Canadian national data are available for children less than 6 years of age.

There are no recent nationally representative data on blood lead concentrations in Canadian children less than 6 years old; however, estimates of the blood lead concentrations in Canadian children less than 6 years old can be made by at least two methods.

There are no nationally representative, recent data on blood lead concentrations in Canadian children less than 6 years old. Data from us national biomonitoring studies provides a reasonable proxy for Canadian blood lead distributions. Unfortunately, the most recently available published U.S. NHANES data is from 2003-04¹² and the mean blood lead concentration in children may have declined since then.

Alternatively, estimates of the distribution of blood lead concentrations in Canadian children less than 6 years old can be made by: (1) estimating the geometric mean blood lead in children based on the most recent estimate of the geometric mean blood lead concentrations in Canadians 20-69 years old; (2) estimating the geometric standard deviation of blood lead concentrations in Canadian children less

¹² Raw NHANES blood lead data is available for as recently as 2007-08, but a relatively complex procedure is required to correct the data for the sample weightings of the US NHANES study design.

than 6 years old based on the reported mean and 95th percentile blood lead concentration in U.S. children in the most recently published US NHANES results; and (3) using the estimated geometric mean and standard deviation to derive an estimate of the 95th percentile blood lead concentration in Canadian children less than 6 years old.

In recent U.S. national biomonitoring studies the mean blood lead concentrations among adults has been similar to (within the 95th percent confidence intervals), to slightly lower (+0.2 μ g/dL) than the mean blood lead concentrations in children 1-5 years old (US CDC, 2009). Subjects aged 6 to 19 years old tend to have lower blood lead concentrations than the younger and older life-stages. Recent estimates of the mean blood lead concentrations in adult Canadians may be used to estimate the mean blood lead concentrations in Canadian children less than 6 years old. By this method, the geometric mean blood lead in Canadian children is estimated to be about 1.7 μ g/dL (1.5 + 0.2 = 1.7).

While the mean blood lead of children less than 6 years old has tended to be similar or just slightly higher than the mean blood lead concentration among adults, the variance in blood lead concentration in children less than 6 years old has tended to be greater than that of adults. Therefore, the 95th percentile blood lead concentration in children less than 6 years old also tends to be higher than the 95th percentile blood lead concentration among adults. We used the reported mean and 95th percentile blood lead concentration in U.S. children 1-5 years old from the most recently published US NHANES results (2003-04) to calculate a geometric standard deviation for the blood lead concentration in children 1-5 years old (GSD = $e^{(ln(5.10)-ln(1.77))/1.6449)} = 1.9 \,\mu\text{g/dL}$).

Finally, the geometric standard deviation (1.9 μ g/dL) estimated from the published 2003-04 US NHANES percentiles and the geometric mean (1.7 μ g/dL) estimated

from the 2007-08 CHMS mean blood lead concentration in Canadian adults can be used to estimate a 95th percentile blood lead concentration in Canadian children less than 6 years old of 4.9 μ g/dL (95th percentile = e^{(ln(1.7) + 1.6449 × ln(1.9))}. Given the uncertainty in the estimate, it is more appropriate to define the estimate as approximately 4-6 μ g/dL.

Therefore, the distribution of blood lead concentrations in Canadian children less than 6 years old can be estimated to have a geometric mean of about 1.7 μ g/dL and a 95th percentile of about 4-6 μ g/dL.

Data on the distribution of blood lead concentrations in Canadians can be used to define the range of blood lead concentrations over which estimates of exposure-response relationships should be most robust, as this will be the range of blood lead concentrations of those who are currently exposed. Based on the data above, it is estimated that more than 70% of Canadians have blood lead concentrations between 1 and 5 μ g/dL. Because the majority of Canadians have blood lead concentrations within which this report attempts to derive the most certain estimates of the blood lead concentration-response relationships for IQ and SBP.

Rationale for the Selection of Critical Studies

An empirical, rather than mechanistic, approach was used to derive quantitative estimates of the blood lead concentration-response relationships. The empirical approach requires: (1) the selection of study data upon which to base the estimate; and (2) the selection of a model to represent the study data. The studies upon which the quantitative blood lead concentration-response estimates are based are defined as *critical studies*. Rationale is provided in the following sections for selection of the critical studies, as well as for the selection of the blood lead concentration-response model for each of the endpoints.

General considerations and criteria for selecting published blood lead concentrationresponse models are presented below. Furthermore, details of the selection rationale and the selected model for each of the endpoints are provided in the sections that follow.

Inter-study variability is a source of uncertainty in the estimated blood lead concentration-response relationships. For both IQ and SBP there is considerable variability in the reported blood lead concentration-response relationships, including some studies that report no statistically significant relationship (i.e., the slope of the relationship is not significantly different than zero). The inter-study variability in reported blood lead concentration-response relationships is a function of: (1) variability between study cohorts in the underlying blood lead concentration-response relationship; (2) variability among studies in their design and execution and their related power to detect a relationship and the rigour of their methods of reducing bias and confounding; and (3) variability among studies in the functional form of the models used to represent the blood lead concentration-response relationships.

In the context of these sources of inter-study variability, the following criteria were used to guide selection of the critical studies:

- Epidemiological data were preferred over animal data
- Epidemiological studies of environmental cohorts were preferred over occupational cohorts
- Epidemiological studies that took rigorous efforts to control for confounding and bias were preferred over those that did not
- Studies that are representative of the range of contemporary environmental lead exposures in Canada were given priority (this requirement also necessitated selection of more recent studies)

• Studies that were based on data representative of the genetic and social diversity within the Canadian population were given priority

Some referees of an earlier draft of this report recommended using meta-analysis to address inter-study variability in blood lead concentration-response relationships. While meta-analysis may be helpful for deriving a combined estimate of effects from similar studies—except for the studies of children's blood lead and IQ, for which a pooled analysis was available, the available studies are not sufficiently similar to derive a defensible combined blood lead concentration-response estimate. For example; some published meta-analyses have derived a combined effect from both occupational and environmental studies (Nawrot *et al.*, 2002; Navas-Acien *et al.*, 2008).. Rather than using meta-analysis to derive a combined estimate of the blood lead concentration-response relationship, except for blood lead-IQ, the blood lead concentration-response relationship was quantified from multiple studies and presented as a side-by-side comparison of these estimates to illustrate the potential inter-study variability.

Selection of Blood Lead Concentration-Response Models

Another source of uncertainty in the estimated blood lead concentration-response relationship is the choice of model used to represent it. As mentioned above, existing, published blood lead concentration-response models were used, and the objective when selecting existing models of the blood lead concentration-response relationships was to identify models that were, to the extent possible, true to the underlying data (e.g., provided a defensible fit to the data). Published models that were accompanied by evidence of appropriate functional form and goodness of fit were preferred over those that were not.

The selection of critical studies and blood lead concentration-response models for each of the endpoints is discussed in more detail below.

5.3 CHILDREN'S BLOOD LEAD AND IQC IN THE RECOMMENDED SLOPE OF THE BLOOD LEAD CONCENTRATION-RESPONSE RELATIONSHIP

Shape and Extent of the Blood lead Concentration-IQ Response Relationship

A key issue in determining the extent of the blood lead concentration-response relationship is ascertaining what lower limit of chronic blood lead is associated with adverse effects. In other words; can a threshold of effect be identified? Another debated issue is the shape of the blood lead concentration-response relationship at relatively low blood lead concentrations (Bergdahl, 2006; Bowers and Beck, 2006b; Bowers and Beck, 2006a; Jusko *et al.*, 2006; Bergdahl, 2007; Bowers and Beck, 2007b; Bowers and Beck, 2007a; Svendsgaard *et al.*, 2007). This debate arose in response to evidence from Lanphear *et al.* (2005), Canfield *et al.* (2003a), Schwartz (1994a), and others that the slope of the blood lead concentration-response relationship appeared curvilinear and steeper over the lower range of blood lead concentrations examined in these studies. These two issues are explored in more detail in the following sections.

In general, there have been two types of analytical strategies used to elucidate the extent and shape of blood lead concentration-response relationship between earlylife blood lead and developmental neurotoxicity: (1) categorical comparisons; and (2) continuous non-linear modeling.

Categorical comparisons are made when exposures are expressed as categorical variables and comparisons in response are made between exposure categories. Progressive or segmented linear regression analysis is a type of categorical comparison and involves dividing the study subjects into separate bins or categories of blood lead concentrations and conducting multiple linear regression analysis on the blood lead and endpoint relationship for subjects within each of the bins. Categories can be defined so that equal numbers of subjects are either assigned to each bin (e.g., tertiles, quartiles, etc.) or assigned based on specified exposure cutpoints (e.g., blood lead < 5 μ g/dL vs. blood lead \geq 5 μ g/dL). By this method, researchers attempt to demonstrate whether there are differences in the regression coefficients between different exposure ranges or bins. A simpler method of categorical analysis involves testing for differences in group means of the study endpoint between exposure categories. There are two potential issues with categorical comparisons: (1) the results may be dependent on the choice of cutpoints (or, equivalently, the number of bins); and (2) categorizing the data by absolute cut-points can produce produces an unequal number of subjects in each category. As the number of bins or cut-points increases, the number of data within each bin decreases, and consequently, so does the power to detect an effect. While the results of recent studies that employ these methods are presented here for completeness, they do not carry as much weight in this assessment of the shape and extent of the blood lead concentration-IQ relationship as analyses using nonlinear modeling.

Categorical Analysis

Binning involves dividing the study data into discrete, mutually exclusive "bins" that represent differing exposure levels and comparing the results of analysis of each of the binned data to identify patterns of results across differing exposure levels. Cutpoints for the bins may be selected based on: (1) methods that produce an equal number of subjects within each category (tertiles, quartiles, etc.); (2) methods that space the cut-points equally across the range of blood lead concentrations; or (3) methods designed to test policy-relevant hypotheses (e.g., is the relationship significant for only those subjects with blood lead < 10 μ g/dL?). Authors are not

always explicit about their reasons for their choice of cut-points. Nor is it always stated whether the cut-points were selected *a priori*. Methods that result in a differing number of data points within each bin create discrepancies in power between bins and are, therefore, are not afforded the same weighting in this analysis as methods that have an equal number of data points among the bins.

Jusko *et al.* (2008) used a categorical analysis to examine differences in IQ at age 6 among children from the Rochester cohort who had lifetime average, concurrent, and infancy average blood lead of < $5.0 \mu g/dL$, $5.0 to 9.9 \mu g/dL$, or > $10 \mu g/dL$. The authors reported that children with lifetime average or infancy average blood lead $5.0 to 9.9 \mu g/dL$ had significantly lower adjusted IQ (by 4.9 IQ points) than children with lifetime average or infancy average blood lead of < $5.0 \mu g/dL$. The difference in adjusted IQ between children with blood lead $5.0 to 9.9 \mu g/dL$. The difference in adjusted IQ between children with blood lead $5.0 to 9.9 \mu g/dL$ was not significantly different that those with lead > $10 \mu g/dL$. The results of this analysis add evidence to the suggestion that the slope of the blood lead-IQ relationship is steeper across the lower range of contemporary blood lead values.

Schwartz (1994a) conducted a series of analyses to examine the presence of a threshold in the postnatal blood lead-IQ relationship. Firstly, estimates of effect size were examined as a function of the mean blood lead of the respective studies included in his meta-analysis. No trend of decreasing effect size with decreasing study mean blood lead was observed. This is inconsistent with the expected trend if a threshold were, in fact, within the range of study mean blood lead concentrations. Schwartz (1994a) reported the opposite finding—the estimates of effect size tended to increase as the study mean blood lead concentrations decreased. Schwartz (1994a) also analyzed data from the Boston cohort using continuous non-linear modeling; the results of this analysis are discussed below.

In an analysis of data from the Yugoslavia cohort, Wasserman *et al.* (2003) noted a greater adverse effect on IQ between 1st and 2nd quartiles of tibia lead relative to the magnitude of effect noted between 2nd and 3rd and 3rd and 4th quartiles. This same pattern of greater effect at relatively lower concentrations was also observed for lifetime (birth to 10 years) and concurrent blood lead.

Lanphear et al. (2000) report on a series of analyses of the cross-sectional associations between concurrent blood lead and performance on tests of arithmetic skills, reading skills, nonverbal reasoning and short-term memory among 4,853 subjects aged 6 to 16 years of the US Third National Health and Nutrition Examination Survey (NHANES III) (1988-94). The mean blood lead of this study cohort was 1.9 μ g/dL, and approximately 63% of subjects had a blood lead < 2.5 µg/dL. Covariates considered included subject sex, ethnicity, iron status, serum cotinine, geographic region, parental marital status, education, and socioeconomic status. Maternal IQ and a measure of the care-giving environment were not assessed. Lanphear et al. (2000) used a series of analytical approaches to elucidate the lowest range of blood lead over which adverse effects could be observed. First, significant inverse associations between blood lead and all outcomes, after adjusting for covariates, were reported for an analysis including the entire study cohort data. In a second analysis, an inverse relationship was found between covariate adjusted performance on measures of arithmetic skills and reading skills and quartile of blood lead concentration (i.e.; a statistically significant difference was observed between the 1st and 4th quartiles of blood lead; < 1.0 μ g/dL and > 3.0 μ g/dL, respectively). The magnitude of the estimate of the association between blood lead and reading and arithmetic skills was larger when analyses were restricted to children with blood lead concentrations of less than 2.5 µg/dL compared with analyses with all subjects with blood lead concentrations less than 10 µg/dL. Collectively, these results provide evidence of the developmental neurotoxicity of lead down to blood lead concentrations at least as low as approximately 2.5 µg/dL.

Emory et al. (2003) used a categorical analysis to examine the differences in maternal blood lead between different categories of outcome on an infant test of intelligence. This study examined the relationship between maternal blood lead and intelligence at 7 months among 79 African-American infants from Atlanta, Georgia. Maternal blood lead was sampled at 6 to 7 months' gestation and cord blood was collected at delivery. Additional efforts were undertaken to ensure the precision and accuracy of low level (<5 µg/dL) blood lead analysis remained within tolerable laboratory limits. Infant intelligence was assessed with the Fagan Test of Infant Intelligence (FTII), a well-recognized and standardized test of infant intelligence. A limited number of covariates were examined, including maternal education, and subject age at testing, gestational age, and birth weight. The effect of breastfeeding and other potentially important confounders was not included in this analysis. Exclusion criteria included relevant medical complications during gestation or infancy and maternal use of drugs or alcohol. Maternal blood lead concentrations ranged from 0.05 to 3.3 µg/dL with a mean (SD) of 0.72 (0.86) µg/dL. Infants within the lower 5th percentile of the distribution of subject FTII scores had maternal blood lead values (mean (SD) of 1.18 (0.74) µg/dL) that were six times higher than those of the infants that scored in the upper 5th percentile of the distribution of subject FTII scores (mean (SD) maternal blood lead 0.28 (0.34) µg/dL. This difference was statically significant (p < 0.001). The authors reported that the validity of the FTII is greatest at the tails, with those in the lower tail at greatest risk for developmental difficulties latter in life (Emory et al., 2003). The results of this study suggest that maternal blood lead concentrations as low as approximately 1 µg/dL are associated with developmental neurotoxicity. In a previous study Emory et al. (1999) reported that, among 101 neonatal subjects from the same cohort, those in the 4th guartile for maternal blood lead concentration had significantly poorer scores on subtests of motor control and attention from the Brazelton Neonatal Behavior Assessment Scale when compared to those subjects in the 1st quartile for maternal blood lead.

Bellinger and Needleman (2003) reanalyzed data on 48 children from the Boston cohort whose measured blood lead concentrations never exceeded 10 μ g/dL. The association between blood lead at age 2 and IQ decrements at age 10 remained statistically significant, and the slope of the dose-response relationship was steeper for those whose blood lead never exceeded 10 μ g/dL than for the entire cohort (linear regression coefficient of -1.56 (no SE reported) for those with blood lead < 10 μ g/dL vs. -0.58 (0.21) for the entire cohort).

Chiodo et al. (2004) assessed IQ and an extensive series of other neurodevepmental and behavioural endpoints in a cross-sectional study of a cohort of 246 low SES African-American male and female children from inner-city Detroit, Michigan. Lead exposure was quantified as blood lead at 7.5 years of age; neurodevelopmental and behavioural outcomes were assessed concurrently with blood lead. The mean (SD) blood lead of the subjects was 5.4 (3.3) µg/dL. Nineteen variables were considered as potential confounders, but not nutritional status or maternal IQ. Subject IQ was assessed using the Wechsler Intelligence Scales for Children-III (WISC-III). After adjusting for confounders, significant negative associations between concurrent blood lead and full-scale IQ, verbal IQ, and performance IQ were reported. Significant inverse associations were also reported for a number of neurodevelopmental and behavioural outcomes, including teacherreported attention deficits, teacher-reported withdrawn behaviours, information processing speed, and auditory working memory. In a second set of analyses Chiodo et al. (2004) completed a series of regression analysis for four dichotomous lead exposure groups, with cut-points at 3 µg/dL, 5 µg/dL, 7.5 µg/dL and 10 µg/dL. These cut-points were chosen to match those employed by Lanphear et al. (2000). Significant negative associations were reported for more outcomes when the data was truncated at < 5 μ g/dL than when the data was truncated at < 10 μ g/dL. A number of outcomes were significantly associated with lead when the data was

truncated at < 3 μ g/dL, despite the fact that there were only 31 subjects within this range.

Similar segmented linear regression analyses have been performed on data from the Rochester cohort (Canfield *et al.*, 2003a), a cross-sectional study of Mexican first-graders with relatively high blood lead concentrations (Kordas *et al.*, 2006), the two cohorts from Mexico City (Tellez-Rojo *et al.*, 2006) and the international pooled analysis (Lanphear *et al.*, 2005). All of these analyses report statistically steeper adjusted linear regression coefficients for the lower exposure categories.

The results of categorical analyses of the shape of the blood lead concentration-IQ relationship from two studies do not support the trend of an increasingly steeper inverse association at lower blood lead concentrations:

Al-Saleh *et al.* (2001) report on the cross-sectional association between blood lead concentrations and IQ among 532 females aged 6 to 12 years from Riyadh, Saudi Arabia. Psychological intelligence was assessed with the Beery-Visual-Motor Integration (Beery VMI) test and the Test of Non-Verbal Intelligence (TONI). Both are standardized culture and language independent tests. The study endpoints were Beery VMI score, TONI IQ and TONI percentile rank. Covariates assessed included parental education and occupation, as well as household income. The geometric mean blood lead concentrations among subjects was 7.44 μ g/dL. After adjusting for covariates, blood lead concentrations among the full cohort were significantly inversely associated with Beery VMI test scores and TONI percentile rank, but not TONI IQ. When the data were stratified at 9 μ g/dL, only TONI percentile rank was significantly inversely associated with blood lead concentrations for the 368 subjects with blood lead concentrations $\leq 9 \mu$ g/dL, whereas the adjusted regression coefficients were significant for all three endpoints for those subjects with blood lead concentrations $\geq 9 \mu$ g/dL.

Surkan *et al.* (2007) report on a cross-sectional analysis of the association between IQ and concurrent blood Pb concentrations among 389 subjects aged 6 to 10 years old from the New England Children's Amalgam Trial (NECAT). Subjects were predominantly (74%) Caucasian residing in urban Boston, MA or rural Farmington, ME. IQ was measured using the WISC-III. After adjusting for covariates (age, ethnicity, socioeconomic status, and caregiver IQ), subjects with a blood lead concentration of 5 to 10 µg/dL had a significantly lower mean IQ than subjects with a blood lead concentration of 1 to 2 µg/dL. Subjects with a blood lead concentration of 1 to 2 µg/dL, but the difference was not statistically significant (p = 0.941) and the magnitude of the difference was much less than that of the subjects with a blood lead concentration of 5 to 10 µg/dL. Plots of mean IQ by categorical blood lead concentration indicate that there is no association with IQ until blood lead concentrations exceed about 5 µg/dL.

Summary of Evidence from Categorical Analyses

The evidence from the available categorical analyses report an association between developmental neurotoxicity and maternal blood lead of less than 1 μ g/dL, and postnatal average blood lead as low as ~2 μ g/dL. The majority of analyses (Lanphear *et al.*, 2000; Canfield *et al.*, 2003a; Wasserman *et al.*, 2003; Chiodo *et al.*, 2004; Lanphear *et al.*, 2005; Kordas *et al.*, 2006; Tellez-Rojo *et al.*, 2006; Jusko *et al.*, 2008) indicate a steeper blood lead concentration-response relationship over the lower range of postnatal blood lead concentrations examined in these studies. The studies by Surkan *et al.* (2007) and Al-Saleh *et al.* (2001) provide conflicting evidence. The Surkan *et al.* (2007) study is an important exception because it included a relatively large number of study subjects with blood lead concentrations less than 5 μ g/dL. Given the imprecision in measuring exposure and outcomes and

the many factors influencing IQ, some inconsistency among the results of multiple studies is not unexpected.

Continuous Non-Linear Methods

Continuous non-linear models have also been used to examine the extent and shape of the blood lead concentration-IQ relationship. The data analysis typically involves two steps. The first step involves comparing the statistical significance and fit of parametric non-linear models against a linear regression model. Parametric non-linear models that have been tested include log-linear models; quadratic, cubic and higher order polynomial models; and, in one case, a logit (sigmoidal) model. The second step involves applying a non-parametric model to the study data to illustrate the underlying shape of the relationship without requiring the relation to conform to the shape of a particular parametric function. Non-parametric techniques that have been applied to the blood lead-IQ relationship include spline analysis and locally weighted smoothing. While the non-parametric models offer some advantages in illustrating the "true" form of the relationship, statistical inference is not as well developed for the non-parametric models and researches typically revert to a parametric model to provide a more utilitarian description of the blood lead concentration-IQ relationship.

Testing Alternate Parametric Models

Some authors have tested their data for non-linearity by testing the statistical significance of parametric non-linear (log-linear, cubic or quadratic polynomial, or logit) regression models or by testing the respective fit of linear and non-linear models using the *J*-test (Rothenberg and Rothenberg, 2005). Examples of this approach where statistically significant fits of curvilinear models were obtained include data from the international pooled analysis (Lanphear *et al.*, 2005), the

Rochester cohort (Canfield *et al.*, 2003a), data from both of the Detroit crosssectional studies reported by Chiodo *et al.* (2004; 2007), the two-cohort study from Mexico City (Tellez-Rojo *et al.*, 2006), and the Mexico City Prospective Lead Study data on maternal blood lead and child IQ (Schnaas *et al.*, 2006). The log-linear blood lead concentration-response models published by Schnass *et al.* (2006) and Tellez-Rojo *et al.* (2006) are illustrated in Figure 13 and Figure 14 below. In an independent published analysis of the data from the international pooled analysis, Rothenberg *et al.* (2005), confirmed that the log-linear model provided a statistically significant better fit to the data than a linear model. No studies of blood lead concentrations less than 10 µg/dL and IQ or other measures of neurological functioning were identified where tests of non-linearity were been non-significant or did not provide a better fit to the data than linear models.
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Figure 13. From Schnass et al. 2006. Third trimester maternal blood lead concentration and adjusted IQ test scores among 150 Mexican children aged six to ten years old. The regression line shown is the adjusted log-linear model and 95th percent confidence intervals. The J-test indicated that the log-linear model fit the data significantly better than a linear model.

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Figure 14. From Tellez-Rojo et al. (2006). Concurrent blood lead and twelve and twenty-four month test scores on the Bayley Scales of Infant Development II (BSID-II) Mental Development Index (MDI) among 294 children from Mexico City, Mexico. Tellez-Rojo et al. (2006) report that a log-linear model fit their study data significantly better than a linear model.

Non-Parametric, Non-Linear Regression Modeling

Several researchers have used non-parametric or semi-parametric regression techniques, such as local polynomial regression (Lowess or loess) smoothing or spline analysis, to illustrate the shape of the blood lead concentration-response relationships observed in their studies. In these approaches, the study data are used to determine the functional form of the relationship, rather than imposing a specified (parametric) functional form to the data. Both local polynomial regression smoothing and spline analysis are well-accepted techniques for modeling non-linear relationships. Local polynomial regression smoothing fits a polynomial function (quadradic or cubic) to a local region of the data using inverse weighting. Spline analysis involves the construction of piecewise polynomial functions that are joined at a specified number of points called *knots*. Quadradic and cubic splines produce a smoothly curved continuous relationship.

Spline Analysis

Canfield *et al.* (2003) and Lanphear *et al.* (2005) have both used spline analyses to explore the shape and extent of the blood lead concnetration-response relationship for IQ. Both reported no evidence of a threshold of effects and a steeper concentration-response relationship over the lower ranges of blood lead concentrations included in their studies. These analyses are discussed individually below.

Canfield *et al.* (2003) used a covariate adjusted penalized spline model to illustrate a curvilinear relationship between lifetime average blood lead and IQ measured at 3 and 5 years of age. The analysis indicated a decline in IQ of 7.4 points for a lifetime average blood lead concentration of up to 10 μ g/dL, whereas for lifetime average blood lead concentrations between 10 and 30 μ g/dL, a smaller (i.e. more gradual) decrement of ~2.5 IQ points was estimated. The spline model of Canfield *et al.* (2003), illustrated in Figure 15 below, indicates that the blood lead concentration-IQ relationship shows no sign of attenuation down to the lowest lifetime average blood lead concentrations measured in the study.

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Figure 15. From Canfield et al. (2003). Lifetime average blood lead concentration and covariate adjusted IQ test scores among 172 subjects of the Rochester cohort at three and five years of age. The dark line is a penalized spline model of the relationship.

Lanphear *et al.* (2005) also used a covariate adjusted five knot restricted cubic spline model to analyze the shape of the relationship between concurrent blood lead and school-aged IQ in the international pooled analysis. The restricted cubic spline model, illustrated in Figure 16 below, indicates that a log-linear parametric model provides a good fit to the data. The results of the international pooled analysis by Lanphear *et al.* (2005) also provide evidence that the blood lead concentration-response relationship for concurrent blood lead extends at least as low as the minimum concurrent blood lead concentration in the study of 0.5 µg/dL (Hornung, 2009, *pers. com.*)



Figure 16. From Lanphear et al. (2005). Concurrent blood lead and IQ among 1,333 school-aged children included in the international pooled analysis. The solid line is the five knot restricted cubic spline model and the short dashed lines are the 95th percent confidence intervals on the spline model. The long dashed line is the log-linear model, which was the parametric model that best reflected the shape of the blood lead concentration-IQ relationship indicated by the spline model.

Loess Smoothing

Schwartz (1993), Chiodo *et al.* (2004, 2007), Kordas *et al.* (2006) and Jusko *et al.* (2008) have used locally weighted smoothing techniques to explore the shape and extent of the blood lead concnetration-response relationship for IQ or other measures of developmental neurotoxicity. All reported no evidence of a threshold of effects and a steeper concentration-response relationship over the lower ranges of blood lead concentrations included in their studies. These analyses are discussed individually below.

In the first example of this technique applied to early-life blood lead-IQ data, Schwartz (1993) applied loess non-parametric smoothing to the relationship between the adjusted residuals of IQ and the residuals of the 24 month blood lead data from Bellinger *et al.* (1992). This study was selected because it had the lowest cohort blood lead concentrations available at the time. No discontinuity in the blood lead-IQ relationship, or threshold, was identified by this analysis.

Jusko *et al.* (2008), reporting on data from the Rochester cohort, used a generalized additive model (GAM) with loess locally weighted smoothing to illustrate the shape of the relationship between peak blood lead and IQ at 6 years of age. The regression model was statistically significant for both full-scale and performance IQ, and the results of the loess smoothed scatterplot indicate that the blood lead concentration-IQ relationship is steeper over the lower range of peak blood lead (i.e., < 10 μ g/dL) and that this relationship extends down to the lowest peak blood lead concentrations (2.1 μ g/dL).

Chiodo *et al.* (2004) and (2007) provide a series of reports on the cross-sectional association between neurological outcomes and blood lead among school-aged children from Detroit, Michigan. The cohort demographics and study methods of Chiodo *et al.* (2007) are similar to those previously described for Chiodo *et al.* (2004). However, the analysis of Chiodo *et al.* (2007) included 506 subjects, compared to the 246 African Amercian children included in the 2004 stody. In both publications, local polynomial regression smoothing is used to explore the data for the presence of a threshold. No threshold discontinuity was evident for most endpoints, including full-scale IQ and performance IQ. Results for full-scale IQ and teacher scores of subject attention are illustrated in Figure 17 below (note that the x-axis in these panels is on a log scale). The results for these endpoints are considered representative of the results for most other endpoints examined in these publications. For some endpoints, a steeper dose-response curve was evident over

the lower range of lead exposures. The loess smoothing by Chiodo *et al.* (2004), (2007) indicates that the association between blood lead concentrations and neurodevelopmental effects extends as low as the minimum blood lead concentrations among these study cohorts (1 μ g/dL).



Figure 17. From Chido et al. (2004). Spline analysis of cross-sectional relationship between blood lead (note log scale) at 7 years of age and adjusted full-scale IQ (top) and adjusted teacher-reported attention span (bottom) among 264 subjects from Detroit, Michigan. No threshold of effects is evident over the range of blood lead studied.

A similar technique using Lowess smoothing and a similar pattern of results between concurrent blood lead concentration and scores on various tests of cognitive function (not IQ) among first-grade children living near a metal foundry in Torreón, Mexico was reported by Kordas *et al.* (2006). The Lowess smoothing, illustrated in Figure 18 below, indicates a steeper blood lead concentration response over the lower range

of blood lead concentrations, with no discontinuity down to the lowest blood lead concentrations in this study cohort (less than 5 µg/dL).



Figure 18. From Kordas et al. (2006). Scores on the Peabody Picture Vocabulary Test (PPVT) Spanish language version and concurrent blood lead among 532 Mexican 1st Graders. The regression line was produced by Loess smoothing.

Summary of Evidence from Non-Linear Modeling

Alternate parametric non-linear functional forms of the blood lead concentration-IQ relationship have been tested on data from several cohort studies with relatively low blood lead concentrations, and in all cases, a non-linear model was reported as significant. Non-parametric non-linear modeling of the blood lead concentration-IQ relationship has been conducted on data from the Boston, Rochester, and Detroit cohorts, as well as on the data from the international pooled analysis. In all cases, the non-parametric non-linear models indicate a steeper concentration-response over the lower range of study data and do not indicate the presence of a

discontinuity or threshold for effects. Collectively, these analyses provide evidence that the inverse association between blood lead concentration and IQ extends at least as low as 1-2 μ g/dL. One study indicates that the relationship extends as low as 0.5 μ g/dL concurrent blood lead.

The Plausibility of a Supralinear Blood Lead-IQ Relationship

Bowers *et al.* (2006b) suggest that the reported supralinear blood lead-IQ relationships are statistical artefacts arising from the linear regression of a log-normally distributed independent variable against a normally distributed dependent variable. While the merits of this point of view have been substantially dismissed by other lines of reasoning (Bergdahl, 2006; Jusko *et al.*, 2006; Svendsgaard *et al.*, 2007), the blood lead concentration-response curves produced by spline analysis and local polynomial regression smoothing should be immune to this potential problem.

In addition to substantial epidemiological evidence of a curvilinear blood lead concentration-response curve between early-life lead exposure and developmental neurotoxicity, there are plausible biological explanations for this pattern of biomarker-response relationship. The potential mechanisms for the developmental neurotoxicity of lead are reviewed in detail in Section 3.4. There are multiple plausible mechanisms and the combined outcome of multiple overlapping dose-response relationship. There are also a number of endpoints at the sub-cellular, cellular, and whole-organism level that have been demonstrated to have U-shaped dose responses, with inhibitory and stimulatory effects observed on the same endpoint at differing exposure levels. For example, Patel *et al.* (2006) reported significant inverse associations between cord blood lead and adjusted Brazelton's Neonatal Behavioral Assessment Scale (NBAS) scores among 176 neonates.

However, the domains adversely affected differed between those neonates with cord blood lead < 10 μ g/dL and lead > 10 μ g/dL. Pb²⁺ stimulates the second messenger protein kinase C (PKC) *in vitro* at low picomolar concentrations and inhibits PKC *in vitro* at micromolar concentrations (Tomsig and Suszkiw, 1995). A curvilinear doseresponse is consistent with the dependence of the toxic outcome on some saturable or energy or substrate-limiting process, such as ligand binding or active ion transport. Interaction with multiple receptors with differing capacities and binding affinities can also explain a net curvilinear dose-response. For these reasons, the *a priori* argument that a curvilinear dose-response relationship is not biologically plausible was rejected.

Summary of Epidemiological Evidence of the Extent and Shape of the Blood Lead-IQ Relationship

The collective evidence from the analyses summarized above suggests that the inverse association between early-life lead exposure and developmental neurotoxicity extends to the lower range of blood lead concentrations reported in these studies. There is little evidence of a threshold above the lower range of blood lead concentrations reported in these studies and what evidence that does exist is limited to the results of categorical analyses from cross-sectional studies. But, due to the methodological limitations of these approaches, this evidence is given less weight. Nonetheless, it is important to note that one of the negative studies, that of Surkan *et al.* (2007), appears to have the largest number of study subjects with blood lead concentrations less than 5 μ g/dL and also shows no inverse relationship between blood lead and IQ at blood lead concentrations and children's scores on tests of psychometric intelligence and that this relationship extends down to blood lead concentrations within the current range of most Canadian children (1 to

4 μ g/dL). Non-linear modeling from multiple study cohorts demonstrates that the concurrent blood lead concentration-IQ relationships extends as low as 1 to 2 μ g/dL and one publication shows this relationship extending to as low as 0.5 μ g/dL. Categorical analysis provides supporting evidence and a study using this approach demonstrates a significant adverse effect on tests of fetal intelligence as maternal blood lead concentrations rise from 0.28 to 1.18 μ g/dL. The preponderance of evidence also demonstrates that the slope of the blood lead-IQ relationship becomes steeper at blood lead concentrations less than about 7.5 to 10 μ g/dL.

Critical Study

The international pooled analysis by Lanphear *et al.* (2005) was selected as the critical study for the children's blood lead concentration-response relationship. This study was selected by the following reasons:

- Of the existing longitudinal publications, Lanphear *et al.* (2005) has, by far, the highest number of and greatest diversity of subjects. The analysis included seven of the eight longitudinal data sets on children's blood lead and IQ available at the time of the analysis. The demographic characteristics of the individual study cohorts included in the pooled analysis span a wide range of ethnic, social, and economic variability and, of the available studies, best reflects the ethnic and social diversity of the Canadian population.
- The study examined the potential influence of 10 covariates, and data on these covariates were available for a high proportion of subjects (84%). One potentially important covariate that was not included in the Lanphear *et al.* (2005) pooled analysis is a direct measure of household wealth. However, the pooled analysis did include an analysis of maternal age at delivery, maternal IQ, maternal education at delivery and ethnicity, all of which are correlated with SES.

- The data were from prospective studies with serial blood measurements. It was demonstrated that higher, unmeasured blood lead concentrations from early life exposures were not driving the observed results.
- The authors of the paper provided evidence to justify their selection of concurrent blood lead as the primary lead exposure index.
- An independent published analysis of the pooled data verified that the functional form of the model used in the critical study provided a better fit to the study data than alternate models.
- Evidence was provided in the original publication and in subsequent correspondence with the study authors that the selected functional forms of the models provided better fits to the underlying study data than alternate forms.
- Model stability and sensitivity was assessed, and evidence was provided to demonstrate that the results of the pooled analysis were not overly dependent on the data from any single study, nor were the results significantly affected by exclusion of potential outlier data.

The international pooled analysis by Lanphear *et al.* (2005) was selected as the critical study to represent the blood lead-IQ relationship because of its superior statistical power, diversity of subjects, and rigour of model fitting and testing.

It should be noted that the Lanphear *et al.* (2005) publication contains some errors in reporting the 5th and 95th percentiles of the concurrent blood lead data:There are discrepancies between Tables 1 and 4 and the text for the reported 5th and 95th percentiles of the data for concurrent and lifetime blood lead, with the values reported in Table 1 of the paper at odds with those reported in the text and in Table 4. Dr. Lanphear subsequently reported in an email to the US EPA (US EPA, 2007a) that the values reported in Table 1 of the paper at of the paper are the correct values. The 5th and

95th percentiles of the data for concurrent blood lead reported herein are based on the values in Table 1 of Lanphear *et al.* (2005).

Modeling the Blood Lead Concentration-Response Relationship

Metric

The critical study for blood lead and IQ is a pooled analysis of longitudinal data and, therefore, the blood lead concentrations of subjects were measured on multiple occasions. The longitudinal studies of blood lead and IQ consistently report that the effect size is dependent on the blood lead index. Some studies, such as the Boston cohort (Bellinger *et al.*, 1992), report that early life blood lead measurements correlate better with IQ than latter blood lead measurements or blood lead averaged over a specific period. Others studies report a stronger correlation between the most recent, or concurrent, blood lead measurement and IQ. This latter finding, as reported by Chen *et al.* (2005) for the TLC cohort, is illustrated in Figure 19. In this cohort, the blood lead-IQ relationship became stronger over time, even as absolute blood lead concentrations declined and the strongest relationship between IQ and blood lead was for blood lead and IQ measured at 7 years of age.



Figure 19. Reproduced from Chen et al. (2005). IQ test scores at 2 years, 5 years and 7 years by prior to concurrent blood lead concentration. Data are grouped by blood lead quartile. The Y-value corresponds to the mean test score of the group; the x value corresponds to the median blood lead of each quartile.

Lanphear *et al.* (2005) examined the relationship between IQ and the following four blood lead indices: (1) *early childhood*, defined as the mean blood lead from 6 to 24 months of age; (2) *peak*, defined as the maximum measured blood lead measured any time before the IQ test; (3) *lifetime average,* defined as the mean blood lead from 6 months to the concurrent; and (4) *concurrent*, defined as the blood lead

measured closest in time to the IQ test. Concurrent blood lead had the strongest relationship to IQ, as measured by R^2 , and was, therefore, selected as the preferred blood lead index in their study. The adjusted regression coefficients for the other blood lead indices varied by up to about 10% greater and up to about 25% lower than the adjusted regression coefficient for concurrent blood lead. While concurrent blood lead explained the greatest variance in IQ, the slope of the blood lead were steeper than that of concurrent blood lead.

Concurrent blood lead explained the greatest variance in IQ among the subjects of the pooled analysis and, therefore, concurrent blood lead was the exposure index selected for the recommended blood lead-IQ exposure response relationship. While the regression coefficients for other blood lead indices differed by up to 25%, they are within the overall range of uncertainty in the slope of the blood lead concentration-IQ relationship. Should they be required by risk assessors, the slope for concurrent blood lead also provides a reasonable estimate of the slope for the other blood lead indices (early childhood, peak, and lifetime average).

Functional Form and Extent of the Relationship

Lanphear *et al.* (2005) published two regression models that could potentially form the basis of the recommended slope of the blood lead concentration-IQ relationship: (1) a log-linear regression model based on the entire pooled data set; and (2) a linear regression model based on the subset of subjects from the pooled data that had a maximum blood lead concentration of less than 7.5 μ g/dL. Another practical matter to be addressed is the range of blood lead concentrations over which the recommended slope is valid. These issues are discussed below.

Hornung (2009 *pers. com.*) reported that the minimum concurrent blood lead concentration in the international pooled analysis was 0.5 μ g/dL. Based on a weight of evidence review above, it was concluded that the inverse association between blood lead and IQ extends as low as the range of blood lead concentrations yet studied. Therefore, for risk assessments of blood lead concentrations greater than 1 μ g/dL, the lead concentration-IQ relationship as reported by Lanphear *et al.* (2005) is: (1) within the range of data from the critical study; and (2) is supported by the overall weight of evidence from the broader literature. Guidance for risk assessments of blood lead concentration 7.

Lanphear *et al.* (2005) provide evidence from several lines of analysis that the shape of the blood lead concentration-response relationship of their data is supralinear (non-linear with a steeper blood lead concentration-response relationship evident at lower blood lead concentrations). This is consistent with findings from other longitudinal and cross-sectional studies of children's blood lead and IQ (see Section 3.1 for a detailed discussion of the evidence).

Lanphear *et al.* (2005) used a five knot restricted cubic spline model to examine the shape of the blood lead concentration-response relationship of their data. The spline analysis indicated that the relationship between concurrent blood lead and IQ was supralinear. Multiple linear regression was used to model the relationship between blood lead and children's IQ. Quadratic and cubic terms were added to the linear regression model. Both the quadratic and cubic terms were statistically significant (p < 0.001 and p = 0.003, respectively), providing further evidence that the blood lead concentration-response relationship was non-linear. A log-linear model was adopted by the study authors as the functional form of the relationship that best matched the shape of the blood lead concentration-response relationship for the relationship indicated by the spline model. The reported slope (and 95th percent confidence intervals) for

the covariate adjusted log-linear model between concurrent blood lead and children's IQ over the full range of the pooled data is -2.70 (-3.74, -1.66).

A second, independent analysis of the pooled data (Rothenberg and Rothenberg, 2005) confirmed that the log-linear functional form of the blood lead-IQ relationship provided an appropriate fit to the data and that there is no evidence this functional form of the blood lead-IQ relationship is a product of misspecification of the functional form of confounding variables included in the model. The authors also fit a logit and a third order polynomial function to the pooled data. The logit model has a sigmoidal form and can accommodate a low dose threshold or reduction in the slope of the blood lead concentration-response relationship. These alternate non-linear specifications of the functional form fit the data no better than the log-linear model (the variance in IQ accounted for by blood lead differed by less than 0.2% between the three non-linear specifications of the model).

Lanphear *et al.* (2005) also derived two-piece linear regression models of their data, adjusted for the same covariates as the full log-linear model, with *a priori* defined cut-points. The two-piece linear models were constructed to investigate the possibility of a steeper blood lead concentration-response relationship over lower regions of the pooled data. In one analysis, the data was stratified at a peak blood lead of 7.5 μ g/dL, and in a second similar analysis, the data was stratified at a peak blood lead concentration of 10 μ g/dL. The regression coefficient for the 103 subjects with a peak blood lead concentrations less than 7.5 μ g/dL was significantly different (*p* = 0.015) than the coefficient for the 1,230 subjects with peak blood lead concentrations greater than or equal to 7.5 μ g/dL. No significant difference (*p* = 0.103) was reported between the coefficients for the linear regression models based on a cut-point of 10 μ g/dL. The adjusted regression coefficient (and 95th percent confidence intervals) for the linear model for subjects with a peak blood lead of less than 7.5 μ g/dL was -2.94 (-0.17 to -5.16).

The slopes of the linear regression model and the log-linear model are illustrated in Figure 20 below. The intercepts for the models illustrated in Figure 20 were provided by Hornung (2009, *pers. com.*).



Figure 20. Adjusted regression models of concurrent blood lead and full-scale IQ from Lanphear et al. (2005). Dashed line is adjusted log-linear model for all subjects. Solid line is adjusted linear model for subset of subjects with peak blood lead concentration less than 7.5 μ g/dL. Intercepts of the models were provided by Hornung (2009, pers. com.). Models are illustrated over the range of concurrent blood lead concentrations from which they were derived. Note that the linear model should not be interpreted to mean that blood lead concentrations less than about 2.5 μ g/dL have a positive effect on IQ.

Hornung (2009, *pers. com.*) reported that for subjects with peak blood lead concentrations less than 7.5 μ g/dL, an adjusted linear model fit the data slightly better than an adjusted log-linear model (R^2 of 0.574 vs. 0.572) and that the addition of a quadratic term to the linear model was not significantly different from the linear model (p = 0.368). This indicated that a linear model provided a superior fit to the

data within this range. Hornung (2009, *pers. com.*) also reported that the range of concurrent blood lead concentrations upon which this model was based was 0.5 μ g/dL to 7.4 μ g/dL. Lanphear *et al.* (2005) report that, of the 103 subjects included in this strata, 69 were from the Rochester cohort, 13 from the Boston cohort, 11 from the Yugoslavia cohort, eight from the Mexican cohort, and one from both the Cincinnati and Cleveland cohorts.

While the constant slope of the linear model for subjects with a peak blood lead of less than 7.5 µg/dL would make risk assessments based on this slope less computationally complex, the linear model is also less scientifically defensible than the log-linear model based on the entire pooled data: The linear model was based on less than 10% of subjects included in the pooled analysis and this subset of subjects was comprised primarily of those from the Rochester cohort. While Lanphear et al. (2005) provided results of regression diagnostics and sensitivity analysis for the log-linear model based on the full data set, no similar supporting evidence was published for the low-exposure linear model. This raises questions about the degree to which the low-exposure linear model might have been overly dependent on the data from the Rochester cohort and how representative these data are for the full spectrum of ethnic, economic, and social diversity of the Canadian population. The use of the linear model has the added shortcoming that it will tend to over-estimate the blood lead concentration-response relationship over the higher range of blood lead concentrations (e.g., from about 4 to 7.5 µg/dL). Finally, the slope of the low-exposure linear model is also dependent on the choice of cut-point. This is illustrated by the difference in slopes between the linear model for subjects with peak blood lead less than 7.5 μ g/dL ($\beta_1 = -2.94$) and the linear model for subjects with peak blood lead less than 10 μ g/dL (β_1 = -0.80) (Lanphear *et al.*, 2005). For these reasons, a lower degree of confidence was given to the linear model than the models based on the entire set of pooled data.

Based on the above considerations, it is concluded that the best available estimate of the blood lead concentration-IQ relationship is the adjusted regression coefficient of the log-linear model of the full data set from the international pooled analysis of Lanphear *et al.* (2005). The slope of this relationship is -2.7 IQ points per μ g/dL natural log increase in concurrent blood lead. The range of study data that this slope is based on extends as low as 0.5 μ g/dL, but the results have only been replicated in independent studies at blood lead concentrations as low as 1 μ g/dL. It is therefore recommend that the slope be considered valid for blood lead concentrations \geq 1 μ g/dL. Recommendations for risk assessments of blood lead concentrations less than 1 μ g/dL are provided in Section 7.

Uncertainties & Variability in the Blood Lead-IQ Relationship

This section discusses the uncertainties and variability associated with the recommended blood lead concentration-response relationship for children's blood lead and IQ.

There are several known sources of variability in the slope of the estimated blood lead concentration-response relationship between population mean IQ and blood lead in children. These include:

- The blood lead index used to estimate exposure
- The relative timing, absolute age, and form of the IQ test
- The demographic composition of the exposed population

The slope of the relationship between children's IQ and blood lead is dependent upon the blood lead index and the relative timing, absolute age, and form of the administered IQ test. The concentration response-relationship adopted for this report is based on concurrent blood lead and full-scale IQ, as measured by an age- and language-appropriate version of the Wechsler Intelligence Scales for Children, between about 5 to 10 years of age. To the extent that users of the recommended slope of the blood lead-IQ relationship are applying this slope to different measures of lead exposure, different measures of outcome, or different ages of exposure or outcome, the users should acknowledge the potential uncertainty in the extrapolation of the slope.

Risk assessors and risk managers need to be aware of these sources of variability and ensure that they are accounted for in the application of the blood lead concentration-response relationship recommended herein.

Other sources of uncertainty and variability associated with the recommended blood lead concentration-response relationship for children's blood lead and IQ include:

- Variability among the published studies on the slope of the relationship.
- Uncertainty about what model best describes the underlying relationship.
- Uncertainty in the parameters of the model fit to the data of the critical study.
- Uncertainty about the influence of bias and confounding.
- Inter-individual variability in susceptibility and the resulting variance in the slope of the blood lead concentration-response relationship.

These sources of uncertainty and variability are discussed in more detail below. It is noted and emphasized that the only source of uncertainty and variability that is quantified for the blood lead concentration-IQ relationship recommended by this report is the uncertainty in the slope of the model fit to the study data.

Inter-Study Variance in the Reported Slope of Blood Lead Concentration-IQ Relationship There is variability among the available studies in the adjusted regression coefficients of the reported associations between blood lead and IQ. This inter-study variance can, to some extent, be viewed as a potential indication of the variance in susceptibility amongst the various study cohorts. This is confounded, however, by different study designs and different lead exposure ranges among the studies.

Table 16 below presents a summary of published covariate adjusted regression coefficients of the relationship between blood lead concentration and IQ. The adjusted regression coefficients are presented, as well as a comparison of the change in population mean IQ that is predicted by the respective regression coefficients over the blood lead interval of 1 to 4 μ g/dL.

With the exception of the study by Schnass *et al.* (2006), all of the regression coefficients presented in Table 16 are based on blood lead concentrations of less than 10 µg/dL. The mean maternal blood lead concentration in the study by Schnass *et al.* (2006) is 8 µg/dL; however, this study is presented because it is one of the few available studies with statistical evidence to justify using a log-linear model and can be used as a point of comparison for the Lanphear *et al.* (2005) log-linear model. With the exception of Tellez-Rojo *et al.* (2006), all of the regression coefficients were based on IQ as an endpoint. The subjects in the Tellez-Rojo *et al.* (2006) cohort were followed only to two years of age and their intelligence was assessed using the Bayley Scales of Infant Development.

Three publications provided statistical evidence to support using a log-linear regression model. The adjusted log-linear regression coefficient published by Lanphear *et al.* (2005) provides the shallowest slope of the available log-linear models. Unfortunately, the remaining studies are not directly comparable: The study of Schnass *et al.* (2006) was of maternal blood lead concentrations in a cohort with relatively higher lead exposures, and while all of the subjects in the Tellez-Rojo *et al.*

(2006) study had blood lead concentrations less than 10 µg/dL, the measured outcome was not school-aged IQ, but mental development index (MDI) as measured by the Bayley Scales of Infant Development.

Linear regression models are available for five cohorts where the maximum blood lead concentrations were less than 10 µg/dL or where the analysis was based on a subset of subjects with maximum blood lead concentrations of less than 10 µg/dL. There is about a 20-fold variation in the reported adjusted linear regression coefficients. The shallowest reported relationship was for the subset of the subjects from the Detroit cross-sectional study by Chiodo et al. (2004) with a maximum blood lead of less than 7.5 µg/dL. Excluding the results from this cohort, the reported adjusted linear slopes for the blood lead-IQ relationship vary by about a factor of two. The steepest reported relationship was for the subset of the subjects in the international pooled analysis by Lanphear et al. (2005) whose maximum blood lead concentrations were less than 7.5 µg/dL. The slope of the Lanphear et al. (2005) linear model for subjects with a maximum blood lead of less than 7.5 µg/dL is about two-fold steeper than the next largest adjusted regression coefficients. With the exception of the linear models from Lanphear et al. (2005) and Chiodo et al. (2004) for subjects with maximum blood lead concentrations less than 7.5 µg/dL, all other linear slopes for blood lead concentrations less than 10 µg/dL are about -1 to -2 IQ points per µg/dL increase in blood lead concentration. The linear equivalent of the slope of the log-linear model from the full international pooled data set over the range of 1 to 4 µg/dL is -1 to -2 IQ points per µg/dL increase in blood lead concentration. This is in very good agreement with the slopes of most linear models produced by analyses restricted to blood lead concentrations less than 10 µg/dL. Note that this slope (-1 to -2 IQ points per µg/dL increase in blood lead concentration) is about an order of magnitude steeper than the widely guoted 2.5 IQ point loss estimate associated with an increase in blood lead concentration from 10 to 20 µg/dL that was derived by the 1993 meta-analysis of Schwartz.

Using the recommended slope of the relationship between concurrent blood lead and IQ derived from Lanphear *et al.* (2005) of -2.7 (95% confidence intervals: -1.66 to -3.74) per natural log increase in μ g/dL, an increase in blood lead produces an estimated change in mean population IQ of -3.7 IQ points as blood lead increases from 1 to 4 μ g/dL (about the 25th to 95th percentile of blood lead concentrations in Canadian children). The 95% confidence intervals around this estimate, a decrement in mean population IQ of -2.3 to -5.2 IQ points, contain the estimates that would be produced using all other published adjusted regression coefficients presented in Table 16, except those of Lanphear *et al.* 2005 and Chiodo *et al.* 2004 for the subjects in their respective studies with maximal blood lead concentrations of less than 7.5 μ g/dL.

There is no obvious explanation for the discrepancy in the slopes of the low blood lead concentration (< 7.5 µg/dL) linear models of Lanphear et al. (2005) and Chiodo et al. (2004). The design, analytical approach, number of subjects, and cohort demographics between these two analyses are grossly comparable. The balance of the available analyses are in relatively good agreement that the slope of the blood lead-IQ relationship over the range of blood lead concentrations from about 1 to 10 µg/dL lies somewhere between the two extremes presented by the linear models of Lanphear et al. (2005) and Chiodo et al. (2004) for subjects with maximum blood lead concentrations less than 7.5 µg/dL. On balance, the weight of evidence from analyses of the blood lead concentration-IQ relationship over blood lead concentrations less than 10 µg/dL is supportive of the magnitude of the slope of the relationship as represented by the adjusted log-linear regression coefficient from the analysis of the full data set from the international pooled analysis by Lanphear et al. (2005). It should be emphasized, however, that the results of some analyses suggest that the slope of the relationship could be steeper and some suggest that the slope could be shallower and that the recommended upper and lower

quantitative estimates of the recommended slope of the relationship only partially reflect the variance in the reported slopes of the relationship.

			Age				Estimated delta	
			Outcome	Blood Pb		Adjusted β1 (95%	IQ over blood Pb	
Cohort	n	Outcome	Assessed	measure	Model	Cls)	1 to 4 μg/dL	Reference
Log-linear models								
International Pooled Analysis	1,333	IQ	~5 yrs to 10 yrs	Concurrent	Log-linear model of all data	-2.70 (-1.66 to - 3.74)	-3.7 (-2.3 to -5.2)	Lanphear <i>et al.</i> 2005
2 Cohort Study, Mexico City, Mexico	294	Translated BMDI	2 yrs	Concurrent	Log-linear	-4.0 (no CIs reported)	-5.5	Tellez-Rojo <i>et</i> <i>al.</i> 2006
Mexico City Prospective Lead Study	150	IQ (Translated WISC-R)	6-10 yrs	3 rd Trimester Maternal	Log-linear	-3.90 (-1.36 to - 6.45)	-5.4 (-1.9 to -8.9)	Schnass <i>et al.</i> 2006
Linear models								
International Pooled Analysis	244	IQ	~5 yrs to 10 yrs	Concurrent	Linear < for subjects with peak < 10 µg/dL	-0.80 (-0.14 to - 1.74)	-2.4 (-0.4 to -5.2)	Lanphear <i>et al.</i> 2005
International Pooled Analysis	103	IQ	~5 yrs to 10 yrs	Concurrent	Linear < for subjects with peak < 7.5 µg/dL	-2.94 (-0.71 to - 5.16)	-8.8 (-2.1 to -15.5)	Lanphear <i>et al.</i> 2006
Rochester, NY USA	172	IQ (S-BIS)	3 & 5 yrs	Lifetime average	Linear estimate over 1 to 10 µg/dL based on penalized spline model of full sample	-0.8 (no Cls available from spline model)	-2.4	Canfield <i>et al.</i> 2003
2 Cohort Study, Mexico City, Mexico	294	Translated BMDI	2 yrs	Concurrent	Linear estimate over 1 to 10 µg/dL from log-linear model of all data	-1.0	-3.0	Tellez-Rojo <i>et</i> <i>al.</i> 2006
2 Cohort Study, Mexico City, Mexico	294	Translated BMDI	2 yrs	Concurrent	linear	-1.04 (no Cls reported)	-3.1	Tellez-Rojo <i>et</i> <i>al.</i> 2006
2 Cohort Study, Mexico City, Mexico	294	Translated BMDI	2 yrs	Concurrent	Linear < for subjects with peak < 5 µg/dL	-1.71 (no Cls reported)	-5.1	Tellez-Rojo <i>et</i> <i>al.</i> 2006

Table 16. Summary of adjusted blood lead-IQ regression coefficients from studies of subjects with blood lead concentrations less than 10 µg/dL.

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			Age				Estimated delta	
			Outcome	Blood Pb		Adjusted β1 (95%	IQ over blood Pb	
Cohort	n	Outcome	Assessed	measure	Model	Cls)	1 to 4 μg/dL	Reference
Boston, MA USA	48	IQ (WISC- R)	10 yrs	2 years	Linear	-1.56 (no Cls reported)	-4.7	Bellinger & Needleman, 2003
Detroit, MI USA	193	IQ (WISC- III)	7.5 yrs	Concurrent	Linear for subjects with concurrent blood Pb < 7.5 μg/dL	-0.14 (no Cls reported)	-0.4	Chiodo <i>et al.</i> 2004

Uncertainty in the choice of model

As discussed above, there is uncertainty in the functional form of the model fit to the study data. There is considerable scatter in the study data (see Figure 1 in Rothenberg and Rothenberg (2005)) which reduces the ability to identify a model that best represents the pattern in the data. Under conditions of considerable scatter, several models may fit the data almost equally well (or poorly). While Lanphear *et al.* (2005) and Rothenberg and Rothenberg (2005) have provided adequate evidence that, of the models tested, the log-linear model provides the best fit to the data, untested models or differing mathematical constructs of the blood lead concentration-response relationship (e.g., structural equation modeling) may provide alternate estimates of the slope of the blood lead concentration-response relationship.

Uncertainty in the slope of the model

Lanphear *et al.* (2005) published 95th percent confidence intervals for the slopes of both the log-linear model based on the full data set and the linear model based on subjects with a peak blood lead of less than 7.5 µg/dL. However, these confidence intervals only account for the uncertainty in the value of the adjusted regression coefficient. They do not account for other sources of uncertainty and variability in the overall estimate of the blood lead concentration-response relationship, such as uncertainty in the choice of model or variability in the magnitude of blood lead concentration-response relationships among sub-populations. Additionally, the 95th percent confidence intervals for the regression coefficients assume that the independent variable (concurrent blood lead) has been measured without error. Random measurement error in the independent variable will be null biasing (e.g., tend to decrease the magnitude of the regression coefficient) and could underestimate the width of the confidence intervals of the regression coefficient (Greene and Ernhart, 1993).

While using the confidence intervals of the slopes of the observed blood lead-IQ relationship to provide a quantitative estimate of the potential uncertainty in the blood lead concentration-response relationship is recommended, caution must be observed with respect to calling them 95th percent confidence intervals as they do not account for all sources of uncertainty. Even within the domain of the uncertainty in the fit of the regression model, they assume that the independent variable was measured without error. Rather, it is recommended that the published confidence intervals on the slopes be used to provide upper and lower estimates of the observed blood lead concentration-response relationship and that risk assessors be explicit that the degree of precision of these upper and lower estimates cannot be quantified. The upper and lower estimates of the blood lead concentration-response relationship. They do not bound the entire range of reported slopes of the blood lead concentration-response relationship. They do not bound there are relatively few data supporting estimates of the blood lead concentration-response relationship, but there are relatively few data supporting estimates of the blood lead concentration-response relationship.

Bias and Confounding

The magnitude of the slope of the relationship between mean population IQ and concurrent blood lead in children is undoubtedly influenced by some unknown degree by confounding (see Section 3.4 for a more detailed discussion of the potential influence of confounding). However, the magnitude of the slope of this relationship is likely attenuated to some unknown degree by over-control of potential confounding variables (Bellinger, 2004). The suggestion that the observed associations between children's blood lead and decrements in IQ are entirely or even largely based on confounding is also rejected as tthere is strong evidence of the developmental neurotoxicity of lead from *in vivo* animal experiments. Furthermore plausible and relevant mechanisms for these effects have been demonstrated at environmentally relevant levels of blood lead concentration. The association between

children's blood lead and IQ has been observed in a diversity of cohorts with varied socioeconomic and ethnic backgrounds and with a diversity of lead exposure sources. If the observed associations are attributable to unmeasured confounding, the confounding variables must be unrelated to socioeconomic status, ethnicity, or lead source. There is no evidence that the observed association from the critical study was unreasonably affected by selection, measurement or other bias. On balance, there is no compelling evidence to support a qualitative or quantitative adjustment to the estimate of the slope of the relationship between mean population IQ and concurrent blood lead in children, beyond that which has already been recommended, to account for the potential influence of bias or confounding.

Variability in Blood Lead Concentration-Response

A critical issue related to potential variability in blood lead concentration response is whether the blood lead concentration-response relationship based on the critical study needs to be quantitatively adjusted to account for potentially sensitive sub-populations.

Several variables may modify the blood lead concentration-IQ relationship (see Section 3.4 for detailed discussion). These include genetics, overall health status, and environmental variables, such as co-exposure to other chemicals or stressors and nutritional status. At the time of this report, no published studies that examined the potential modifying effect of genetic polymorphisms on the blood lead-IQ relationship were located in the literature. There is, however, evidence from an epidemiological study of lead and cognitive function in older male adults that suggests that the neurotoxic effects of lead may be modified by genetic polymorphisms (Wang *et al.*, 2007). In epidemiological studies of early life lead exposure and neurological development, a stronger inverse association has been reported sometimes, but not always, for subjects with lower SES. Animal studies provide strong evidence for interactive effects between lead and stress, but the pattern of effects is complicated and differs in the outcome measured, brain region affected, time-point of assessment and sex.

Conclusions Regarding Variance in Response

While there is some evidence of the potential modifying effects of environmental stress and genetic polymorphisms on the relationship between lead exposure and neurotoxicity, the science is not sufficiently mature to support quantitative adjustments to the recommended slope of the blood lead concentration-IQ relationship or derivation of a second slope to bound the potential variability in exposure-response relationships among susceptible sub-populations. Additionally, the blood lead concentration-IQ relationship for this endpoint was derived from a large international pooled analysis of study populations of diverse ethnicity and SES (Lanphear *et al.*, 2005). The variability in susceptibility among the cohorts included in the Lanphear *et al.* (2005) pooled analysis was judged to be sufficiently representative of the variability in susceptibility that might be expected across the Canadian population. Therefore, quantitative or qualitative adjustments to the recommended slope of the blood lead-IQ relationship to account for variability in blood lead concentration-response among potentially susceptible sub-populations of differing genetic, social, or economic composition are not recommended.

Summary of Uncertainty & Variability in the Blood Lead-IQ Relationship

In summary, there are multiple sources of variability and uncertainty in the estimated slope of the blood lead concentration-IQ relationship. These include:

- Variability in the slope of the relationship among the published studies.
- Uncertainty about what model best describes the underlying relationship.
- Uncertainty in the parameters of the model fit to the data of the critical study.
- Uncertainty about the influence of bias and confounding.
- Inter-individual variability in susceptibility and the resulting variance in the slope of the blood lead concentration-response relationship.

- The blood lead index used to estimate exposure.
- The relative timing, absolute age, and form of the IQ test.

Section of the Critical Study

The use of a pooled analysis as a critical study somewhat attenuates the potential influence of inter-study variability in the reported blood lead concentration-response relationship. However, some important quantitative differences between the slope of the relationship based on the critical study and published results from other analyses were noted: The recommended slope based on the log-linear model of Lanphear *et al.* (2005) is shallower than the two other available analyses where a log-linear functional form of the relationship was demonstrated to be statistically superior to a linear model. The other two log-linear models are not directly comparable, however, because of differences in measures of lead exposure and psychometric intelligence. The recommended slope based on the log-linear model of Lanphear *et al.* (2005) is in close agreement with the adjusted regression coefficients of most linear models based on blood lead concentrations less than 10 µg/dL. There are, however, a few exceptions.

Uncertainty in the Functional Form of the Relationship

While there is some uncertainty in the choice of the functional form of the model that best represents the blood lead-IQ relationship, the preponderance of evidence indicates that the shape of the relationship, over the range that it has been studied, is supralinear with a steeper slope and little evidence of a threshold at relatively low blood lead concentrations.

Bias and Confounding

The observed relationship between blood lead and IQ is unlikely to be entirely attributable to confounding, as there is strong evidence of biological plausibility. This association has been observed in multiple cohorts of divergent ethnic, social and economic demographics, and the association has been repeatedly observed in longitudinal cohort studies. While the magnitude of the slope of the recommended relationship between mean population IQ and concurrent blood lead in children is undoubtedly influenced by some unknown degree by confounding, it is also likely attenuated by over-control. There is no evidence that the observed association from the critical study was unreasonably affected by selection, measurement or other bias.

Variance in Susceptibility

While there is some evidence of the potential modifying effects of environmental stressors and genetic polymorphisms on the relationship between lead exposure and neurotoxicity, the blood lead concentration-IQ relationship for this endpoint was derived from a large international pooled analysis of study populations of diverse ethnicity and socioeconomic status. Therefore, the variability in susceptibility among the cohorts included in the Lanphear *et al.* (2005) pooled analysis was judged to be sufficiently representative of the variability in susceptibility that might be expected across the Canadian population.

Accounting for Variability and Uncertainty

The 95th percent confidence intervals on the adjusted log-linear regression coefficient from the critical study are recommended to partially quantify the potential influence of these sources of variability and uncertainty. However, the confidence intervals are derived only based on the uncertainty in the value of the model parameter (the regression coefficient). They do not quantitatively account for all other sources of uncertainty and variability in the overall estimate of the blood lead concentration-response relationship. The upper and lower

estimates of the blood lead concentration-response relationship represent plausible, but less likely, values for the slope of the relationship. They do not bound the entire range of reported slopes of the blood lead concentration-response relationship, but there are relatively few data supporting estimates of the blood lead concentration-response relationship outside of this range.

Recommended Slope and Sample Calculations

In summary, the recommended slope of the relationship between population mean IQ and concurrent blood lead in children is based on the adjusted regression coefficient of the loglinear model from the critical study by Lanphear et al. (2005). The covariate adjusted loglinear slope of the relationship between IQ and concurrent blood lead in children for the entire pooled data is -2.70 IQ points per natural log µg/dL increase in concurrent blood lead. The reported 95th percent confidence intervals on the log-linear slope are -1.66 to -3.74 IQ points per natural log increase in µg/dL of concurrent blood lead. The minimum and 95th percentile concurrent blood lead from the pooled analysis were 0.5 and 33.2 µg/dL, respectively. While the 95th percent confidence intervals in the slopes from the critical study are used to derive quantitative estimates of the uncertainty and variability in the blood lead concentrationresponse relationship, it is recommended that these slopes be defined more generally as upper and lower estimates of the potential variability and uncertainty in the slope to avoid misunderstandings about the potential precision of the estimates. While the minimum concurrent blood lead concentration from the critical study was 0.5 µg/dL, the observed association has only been independently replicated in studies with blood lead concentrations as low as 1 µg/dL. Therefore, the relationship underlying the slope from the critical study has only been replicated down to concurrent blood lead concentrations of 1 µg/dL. Risk assessments that require an estimate of the blood lead concentration-response relationship for blood lead concentrations less than 1 µg/dL will necessarily be based on extrapolation.

Guidance and recommendations for extrapolation of this relationship below blood lead concentrations of 1 μ g/dL are provided in Section 7.



Figure 21. Recommended slopes of the relationship between concurrent blood lead in children and change in mean IQ. The middle slope represents the best estimate of the relationship and is bounded by an upper and a lower quantitative estimate that reflects some, but not all, of the potential uncertainty and variability in the blood lead concentration-response relationship. The slopes of these relationships are based the maximum likelihood and 95^{th} percent confidence intervals of the slopes of the adjusted blood lead concentration-response models published in the critical study of Lanphear et al. (2005). The minimum range of concurrent blood lead concentrations in the critical study was $0.5 \mu g/dL$, but the study results have only been replicated in other studies down to blood lead concentrations of $1 \mu g/dL$. Therefore the uncertainty in the shape and extent of these slopes greatly increases at blood lead concentrations less than about $1 \mu g/dL$.

The parameters of the recommended blood lead concentration-IQ slopes are presented in Table 17 and the slopes are illustrated in Figure 21 (above). Equations for calculating population mean IQ as a function of concurrent blood lead are presented below. Table 18 presents the results of applying the recommended slopes to a series of hypothetical changes in concurrent blood lead concentrations in children.

Table 17. Maximum likelihood and upper and lower estimates of the slope of the relationship between population mean IQ and concurrent blood lead in children.

Estimate	Slope ¹ of Log-Linear Model
Upper estimate	-1.66
Best estimate	-2.70
Lower estimate	-3.74

1. The slopes for the log-linear components are the reported maximum likelihood and 95th percent confidence intervals of the adjusted log-linear model for all subjects in the Lanphear *et al.* (2005) pooled analysis. The slopes are in units of IQ points per natural log blood lead µg/dL.

Equation 1 is used for calculating estimates of mean population IQ as a function of children's concurrent blood lead concentrations.

Equation 1

 $IQ = \beta_1 \times \ln(BPb)$; $BPb > 1 \,\mu g/dL$

where

 β_1 = adjusted log - linear regression coefficient; the best estimate is - 2.70,

the upper estimate is -1.66 and the lower estimate is 3.74

BPb = blood Pb concentration

Equation 2 is used for calculating estimates of change in mean population IQ (delta IQ) as a function of changes in children's concurrent blood lead concentrations.

Equation 2

$$\Delta IQ = \beta_1 \times \ln \left[\frac{BPb_2}{BPb_1}\right]; BPb > 1 \,\mu g/dL$$

where

 BPb_1 = lower blood Pb concentration BPb_1 = higher blood Pb concentration ΔIQ = delta mean population IQ
x ₁ (µg/dL)	x ₂ (µg/dL)	Delta blood Pb (µg/dL)	Lower estimate of delta IQ points ¹	Best estimate of delta IQ points ¹	Upper estimate of delta IQ points ¹
1.0	4.0	3.0	-2.3	-3.7	-5.2
2.0	5.0	3.0	-1.5	-2.5	-3.4
2.0	4.0	2.0	-1.2	-1.9	-2.6

Table 18. Examples of application of the recommended slopes of the relationship between concurrent blood lead in children and population mean IQ to hypothetical changes in blood lead.

1. Change in population mean IQ is estimated to a tenth of an IQ point, because IQ is reported in the critical study with this degree of precision.

Table 18 presents the results of applying the recommended slopes of the relationship between concurrent blood lead in children to population mean IQ to a series of hypothetical changes in concurrent blood lead concentrations. A change in concurrent blood lead in children from 1.0 μ g/dL to 4.0 μ g/d, which is estimated to be approximately equivalent to the 25th to 95th percentile of blood lead concentrations in Canadian children 5 to 10 years old, is associated with a change in mean IQ among those children of approximately -2.3 to -5.2 IQ points, with a best estimate of -3.7 IQ points. The potential range of IQ loss may be larger because the estimates provided do not account for all potential sources of uncertainty and variability in the blood lead concentration-IQ relationship.



ADULT BLOOD LEAD AND SBP

In this section of the report:

- A rationale for the selection of the critical study of the relationship between blood lead concentrations in children and IQ is presented
- Based on the critical study, a quantitative estimate of the slope of the blood lead concentration-response relationship is recommended
- Sources of uncertainty and variability in the recommended slope of the blood lead concentration-response relationship are reviewed

Critical Studies

Similar to the epidemiological studies of children's blood lead and IQ, there is a great deal of inter-study variability in the reported blood lead concentration-response relationships for adult blood lead and SBP. However, unlike the blood lead-IQ relationship, no published pooled analysis or suitable meta-analysis that provides a combined estimate of the blood lead concentration-response relationship for blood lead and SBP was located at the time of this report. While there are several published meta-analyses in the literature, there is too much heterogeneity among the individual studies included in the meta-analyses for the combined estimate to provide a reasonable estimate of the expected blood lead concentration-response relationship among environmentally exposed Canadians.

Meta-analyses conducted by Nawrot et al. (2002) and Staessen et al. (1994a) included both occupational and environmental cohorts in their combined estimates. The analysis by (Schwartz, 1995) included studies of males only. Individual studies included in all three metaanalyses were highly variable in terms of the magnitude of exposure amongst the subjects and degree to which potential confounding variables, such as age, smoking, diet, and SES were measured and controlled. While all three meta-analyses reported a significant positive association from the combined analysis, the effect size tended to be much smaller than that reported in some individual studies. It is more likely that the variation in response among the individual studies reflects the variability in study methods and cohorts, rather than population variability of biomarker-response relationships. Additionally, the inclusion of occupational cohorts could dampen the relationship, because of the potential influence of the healthy worker effect; and the inclusion of both higher exposed occupational and historic environmental cohorts could attenuate the overall relationship, because animal studies have shown that a stronger relationship between exposure and blood pressure occurs at relatively low exposures. Therefore, accepting a weighted average of blood lead concentrationresponse relationships derived from diverse study results could not be taken as any more

representative than those from individual studies. Rather than rely on a combined blood lead concentration response-relationship derived from dissimilar studies, it was judged more defensible to use blood lead concentration-response relationships from well-designed individual studies of appropriately representative cohorts.

As described in Section 5.1.1, prospective cohort studies were given preference as critical studies. Six longitudinal studies of the association between blood lead and SBP published since 1980 were identified (Weiss *et al.*, 1986; Neri *et al.*, 1988; Moller and Kristensen, 1992; Staessen *et al.*, 1996; Glenn *et al.*, 2003; Glenn *et al.*, 2006). Three of the six longitudinal studies that included SBP as an endpoint reported a significant positive association with blood lead after adjusting for covariates.

None of the longitudinal studies of environmental cohorts reported a significant association between blood lead and SBP after adjusting for potential confounders. It was therefore not possible to select a longitudinal study of an environmental cohort as the critical study for this endpoint.

Two of the three longitudinal studies reporting a significant positive result were from currently occupationally exposed cohorts (Weiss *et al.*, 1986; Glenn *et al.*, 2006); the third positive study, conducted by (Glenn *et al.*, 2003)), was from a formerly occupationally exposed cohort, although the blood and bone lead concentrations in this cohort were comparable to environmentally exposed subjects at the time of the study (mean blood lead at baseline was 4.6 μ g/dL with a standard deviation of 2.6 μ g/dL; mean tibia lead at year three of the study was 14.7 μ g/g with a standard deviation of 9.4 μ g/g). The Glenn *et al.*, 2003 study population was occupationally exposed for 18 years, on average, prior to the study. The association between baseline blood lead and annual change in SBP was stronger for the subset of subjects who had lower past peak tibia lead concentrations than for the whole cohort combined. This suggests that it is not merely higher historical exposures that are driving the

observed relationship between blood lead and SBP. Therefore, the longitudinal study by Glenn *et al.* (2003) best meets the selection criteria for a critical study to estimate the relationship between adult blood lead and SBP among environmentally exposed Canadians.

The subjects in the study by Glenn *et al.* (2003), however, are not adequately representative of the diversity of the Canadian population as all of the predominantly Caucasian subjects in the study of Glenn *et al.* (2003) were relatively healthy men. The mean (standard deviation) age of the subjects at baseline (1994-1996) was 55.8 (7.4) and they had a SBP of 129.3 (13.9) mmHg. In comparison, the mean (standard deviation) blood pressure in male Canadians aged 55 to 64 during the period 1986-92 was 137.3 (17.0) mmHg (Canadian Heart Health Database, 1992).

The evidence of the population variability in susceptibility to the hypertensive effects of lead is reviewed in Section 4.2. In summary, there is evidence to suggest that ethnicity, sex, stress, nutritional status, co-exposure to other xenobiotics, and genetics may all modify the blood lead concentration-response relationship between lead and SBP. For example:

- Glenn *et al.* (2001) reported that the slope of the adjusted blood lead-SBP relationship for the 5% of subjects in their study cohort who were homozygous for a restriction fragment length polymorphism of the sodium-potassium adenosine triphosphatase alpha 2 (ATP1A2) gene was about twelve-fold higher than that for the balance of their cohort. The authors also reported that the variant allele was 1.8 times more prevalent among African-American subjects in their study cohort.
- Vupputuri *et al.* (2003) reported that the slope of the adjusted blood lead-SBP relationship for African-American women was about five-fold higher than that of Caucasian males. Den Hond *et al.* (2002) also reported a higher slope of the adjusted blood lead-SBP relationship for African-American women relative to Caucasian males.

- Peters *et al.* (2007) reported that the slope of the adjusted tibia lead-SBP relationship for subjects of the Normative Aging Study with high levels of self-reported stress was about 2.5 times higher than subjects with low stress.
- Vupputuri *et al.* (2003) also reported that the relationship between blood lead and SBP differed significantly between the sexes; in contrast, however, the meta-analysis of Nawrot *et al.* (2002) reported that the association between blood lead and SBP was similar between the sexes.
- Nash *et al.* (2003) reported that the relationship between blood lead and risk of hypertension was more pronounced in postmenopausal women than premenopausal women.

In the context of the evidence of potential inter-individual variability in the blood lead concentration-response relationship between lead and SBP and the limited representativeness of the study cohort of Glenn *et al.* (2003), it was necessary to include an additional critical study to characterize the upper range of the reported variance in observed blood lead concentration-response relationships for blood lead and SBP. Because no other longitudinal studies reporting positive results were suitably representative of environmentally exposed Canadians (all were from currently occupationally exposed cohorts), the pool of cross-sectional studies of the relationship between blood lead and SBP was used to identify potential critical studies for susceptable subpopulations.

A range of potential slopes of blood lead concentration-response relationships for blood lead and SBP is illustrated in Figure 22. For the purposes of illustration, all slopes were assigned an intercept of zero. The slope of the blood lead-response relationship for the earlier identified critical study of Glenn *et al.* (2003) is shown relative to the slopes of other selected blood lead-SBP relationships. The blood lead-SBP slopes that are illustrated are the published adjusted regression coefficients from the following studies:

- Nawrot *et al.* (2002): the slope of the maximum likelihood estimate of the combined estimate and its upper 95% confidence interval from a meta-analysis of 31 published cross-sectional studies.
- Den Hond *et al.* (2002): the maximum likelihood estimate of the slope of the adjusted relationship between blood lead and SBP among 4,685 Caucasian male subjects of NHANES III (note that this relationship was not statistically significant (*p* = 0.29)).
- Nash *et al.* (2003): the maximum likelihood estimate of the slope of the adjusted relationship between blood lead and SBP among 1,786 perimenopausal female subjects of NHANES III.
- Rothenberg *et al.* (1999): the maximum likelihood estimate of the slope of the adjusted relationship between blood lead and SBP among 1,188 immigrant Latina women in their third trimester of pregnancy.
- Vupputuri *et al.* (2003): the upper 95% confidence interval of the adjusted regression coefficient of the relationship between blood lead and SBP among 2,300 female African-American subjects of NHANES III.



Figure 22. Adapted from Equilibrium Environmental Inc. (2008a). Variations in reported adjusted blood lead-SBP responses from a selection of representative epidemiological studies. Legend: M = male; F = female; V = various ethnicities; C = Caucasian; LA = Latina; A = African-American; BF = best fit of adjusted regression coefficient; U95CI = upper 95% confidence interval of adjusted regression coefficient. Forf the purposes of comparitive illustartion, all slopes were assigned an intercept of zero.

As illustrated in Figure 22, a number of recent epidemiological investigations report a steeper blood lead-SBP relationship than the study of Glenn *et al.* (2003). The slope for African-American women from the study of Vupputuri *et al.* (2003) was judged to be the best available option to use for an upper estimate of the potential variance in the slope of the blood lead-SBP relationship.

While the slopes of the combined estimate from the meta-analysis of Nawrot *et al.* (2002) are an attractive option for obvious reasons, these slopes might actually present an underestimate of the slope of the blood lead-SBP relationship among susceptible subpopulations because of the influence of the potential diluting effect of the inclusion of predominantly or exclusively male cohorts and occupational cohorts in the meta-analysis. None of the candidate upper estimates illustrated in Figure 22 has a slope nearly as steep as those that have been reported for subjects under stress (Peters *et al.*, 2007) or homozygous for the susceptible variant allele of the ATP1A2 gene (Glenn *et al.*, 2001). However, these results have not been replicated, and it would be premature to base a recommended upper estimate of the slope of the blood lead concentration-response relationship on these reported relationships.

The adjusted regression coefficient for the relationship between blood lead and SBP among African-American female subjects of the Vupputuri *et al.* (2003) study represents the steepest slope of the blood lead-SBP relationship for a potentially susceptible sub-population that has been identified as a susceptible sub-population in multiple studies. Den Hond *et al.* (2002) also reported a stronger relationship between blood lead and SBP for African-American women, as compared to Caucasian males. In the absence any other strongly differentiating criteria, the 95% upper confidence limit of the adjusted regression coefficient for the relationship between blood lead and SBP among African-American female subjects of Vupputuri *et al.* (2003) was selected as the upper limit estimate of the slope of the relationship between adult blood lead and SBP.

The critical studies of Glenn *et al.* (2003) and Vupputuri *et al.* (2003) and the associated slopes of the relationship between blood lead and SBP are described in more detail below.

Glenn *et al.* (2003) analyzed the longitudinal association between blood lead concentration and annual change in BP among 496 former and current male employees of a chemicalmanufacturing facility in the Eastern USA. The facility historically produced tetramethyl and tetraethyl lead. The average age at baseline was 55.8 (SD 7.4) years, the average time since occupational lead exposure was 17.7 (11.6) years, and the average blood lead at baseline was 4.6 (2.6) μ g/dL, with a range of 1 to 20 μ g/dL. The mean SBP was not reported by Glenn *et al.* (2003), but in an earlier report by Schwartz *et al.* (2000b) on 543 subjects from the same cohort, a mean (and standard deviation) SBP of 127.9 (15.3) mmHg was reported. Subjects were followed for an average of 2 (range 0.85-3.5) years and BP was assessed three or four times over this follow-up period. Sitting BP was measured using a random zero sphygmomanometer. BP was recorded as the average of three readings to the nearest 2 mmHg taken at five-minute intervals. Generalized estimating equations (GEE) were used to evaluate the relationship between baseline blood lead and annual change in BP, while controlling for other covariates. After adjusting for baseline age, body mass index, antihypertensive medications, smoking, education, technician and the time to each BP measurement, SBP increased at an average annual rate of 0.64 (95% CI: 0.14-1.14) mmHg for every standard deviation (2.6 μ g/dL) increase in baseline blood lead. This is equivalent to an adjusted linear dose-response slope of 0.25 mmHg per year per μ g/dL with 95% confidence intervals of 0.05 to 0.45 mmHg per year per μ g/dL Significant positive associations were also reported between tibia lead and SBP and peak tibia lead and SBP.

To make the longitudinal slope reported by Glenn *et al.* (2003) comparable to the crosssectional relationship reported by Vupputuri *et al.* (2003), slope of the relationship was assumed to be absolute and, therefore, simplified the slope to 0.25 (0.05 to 0.45) mmHg per μ g/dL. This was viewed as a reasonable simplifying assumption since an earlier crosssectional analysis of 543 subjects from the same cohort reported an adjusted linear regression coefficient (and SE) of 0.504 (0.249) mmHg per μ g/dL (Schwartz *et al.*, 2000b) (i.e., the longitudinal slope was within the 95% confidence intervals of the slope of the crosssectional relationship).

It is recommended that the slope of the adjusted regression model from the critical study of Glenn *et al.* (2003) (0.25 mmHg per μ g/dL) be used to represent the best estimate slope of the relationship between adult blood lead and SBP among Caucasian males. It is further recommended that the lower 95th percent confidence interval on the slope of the adjusted

regression model from the critical study of Glenn *et al.* (2003) (0.05 mmHg per μ g/dL) be used to provide a lower estimate of the slope of the relationship between adult blood lead and SBP among Caucasian males and other relatively less susceptible sub-populations.

Vupputuri et al. (2003) reported on the cross-sectional relationship between blood lead and SBP among 14,952 adult (>18 yrs) participants of the Third National Health and Nutrition Examination Survey (1988-94) (NHANES-III). Triplicate blood pressure measurements were made using a mercury sphygmomanometer during a home visit and at a mobile clinic. Subject's blood pressure was calculated as the average of available measurements. Data were collected on the following potential covariates: age, education, body mass index, alcohol consumption, physical activity, dietary intake of sodium and potassium, and total dietary energy intake (as measured by 24-hour dietary recall). No measure of poverty, an important potential confounder, was included. Subjects taking medication for hypertension were excluded from the analysis and analyses were weighted to account for the NHANES III sampling strategy. Multivariate-adjusted linear regression models were used to examine the cross-sectional association between blood lead and BP, stratified by race and ethnicity. After adjusting for covariates, blood lead was significantly associated with higher SBP and DBP among African-American men (n=2,104) and women (n=2,300), but not Caucasians of either sex. The mean blood lead among the African-American women was 3.4 µg/dL with a standard error of 0.1 µg/dL. The minimum blood lead among subjects was 1 µg/dL (Vupputuri, 2009, pers. com.). The mean (SE) age of the African-American women was 42 (±0.4) years and their mean SBP was 122.4 (±0.6) mmHg. Vupputuri et al. (2003) do not report a regression coefficient and SE from their analysis, but instead report that a one standard deviation increase in blood lead (3.3 µg/dL) was associated with an adjusted increase (95th percent confidence intervals) in SBP of 1.55 (0.47 to 2.64) mmHg. The calculated equivalent B₁ and 95th percent confidence intervals are: 0.47 (0.14 to 0.80) mmHg per µg/dL. The regression model was adjusted for age, education, body mass index, use of alcohol, physical activity, sodium, potassium and total calories.

It is recommended that the slope of the adjusted regression model from the critical study of Vupputuri *et al.* (2003) (0.47 mmHg per μ g/dL) be used to represent the best estimate of the slope of the relationship between adult blood lead and SBP among potentially susceptible sub-populations. Potentially susceptible sub-populations include, but are not limited to, African-American women. It is further recommended that the upper 95th percent confidence interval on the slope of the adjusted regression model from the critical study of Vupputuri *et al.* (2003) (0.80 mmHg per μ g/dL) be used to provide an upper estimate of the slope of the relationship between adult SBP among susceptible sub-populations.

A recent cross-sectional study by Martin *et al.* (2006) reported a steeper adjusted slope of the relationship between blood lead concentrations and SBP. The Baltimore Memory Study is a longitudinal study of the determinants of cognitive decline in urban residents of Baltimore, Maryland. Martin *et al.* (2006) conducted a cross-sectional analysis of the associations between blood lead and blood pressure and hypertension among 964 multi-ethnic (55% Caucasian, 40% African-American, 5% other), mixed sex (66% women), older (mean age 59 years) subjects of the Baltimore Memory Study. Environmental lead exposures were relatively low in this cohort; the mean blood lead of subjects was 3.5 (SD: 2.3) μ g/dL. After adjusting for covariates there was a significant positive association between blood lead and SBP. The adjusted regression coefficient from the Martin *et al.* (2006) study is larger than has been reported for other environmental cohorts and indicates that even the upper 95% confidence interval on the adjusted regression coefficient from Vupputuri *et al.* (2003) may be an underestimate of the slope of the relationship between blood lead and SBP for some communities.

The recommended best estimate slopes of the relationship between adult blood lead and SBP and their quantitative upper and lower estimates are illustrated in Figure 23.



Figure 23. Recommended slopes of the relationship between concurrent adult blood lead and population mean systolic blood pressure. The middle slopes represent the best estimates of the relationships for Caucasian males (lower) and more susceptible sub-populations, respectively. The best estimate slopes are bounded by upper and lower quantitative estimates that reflects some, but not all, of the potential uncertainty and variability in the estimated blood lead concentration-response relationship. The slopes of the relationships for Caucasian males are based on the maximum likelihood and 95th percent lower confidence interval of the slopes of the adjusted linear regression models published in the critical study of Glenn et al. (2003). The slopes of the relationships for susceptible sub-populations are based on the maximum likelihood and 95th percent lower confidence interval of the slopes of the relationships for susceptible sub-populations are based on the maximum likelihood and 95th percent of the slopes of the relationships for susceptible sub-populations are based on the maximum likelihood and 95th percent of the slopes of the adjusted linear regression models published in the critical study of Glenn et al. (2003). The slopes of the relationships for susceptible sub-populations are based on the maximum likelihood and 95th percent upper confidence interval of the slopes of the adjusted linear regression models published in the critical study of Vupputuri et al. (2003). The slopes have not been illustrated at blood lead concentrations beyond the lower limits of the data in the critical studies.

Uncertainties & Variability in the Blood Lead-IQ Relationship

This section discusses the uncertainties and variability associated with the recommended blood lead concentration-response relationship for adult blood lead and SBP. These include:

- Variability among the published studies on the slope of the relationship.
- Uncertainty about what model best describes the underlying relationship.

- Uncertainty in the parameters of the model fit to the data of the critical study.
- Uncertainty about the influence of bias and confounding.
- Inter-individual variability in susceptibility and the resulting variance in the slope of the blood lead concentration-response relationship.

Potential variability in the estimated slope of the blood lead concentration-response relationship amongst different studies and susceptible sub-populations has been addressed above. The remaining sources of uncertainty and variability are discussed in more detail below

Shape and Extent of the Blood Lead Concentration-Response Relationship

The functional form of the blood lead concentration-response relationship and the identification of a threshold of effects have been much less intensively researched for SBP than IQ. Therefore, there is much greater uncertainty in the lower extent and the functional form of the blood lead concentration-response relationship for blood lead-SBP than for blood lead and IQ.

The literature search and review revealed few publications that explicitly tested the shape of the blood lead concentration-response relationship between blood lead and SBP or attempted to identify a threshold for the hypertensive effects of lead. The evidence of a potential non-linear blood lead concentration-response relationship is discussed below. Overall, the published analyses are equivocal. Older studies at higher blood lead concentration to the potential presence of a threshold at about 5 μ g/dL. Other studies are suggestive of effects below this point, but they have not conducted as rigorous an analysis. The epidemiological evidence should be considered in the context of evidence from *in vivo*

experiments, where blood lead concentrations in rats as low as 2.4 μ g/dL have been shown to induce elevated blood pressure.

The strongest relevant evidence on the shape and the extent of the blood lead concentration-SBP relationship comes from analyses by Schwartz *et al.* (2000b) and Martin *et al.* (2006). Both of these analyses point to the possible discontinuity in the blood lead-SBP relationship at a blood lead concentration of about 5 μ g/dL.

Schwartz et al. (2000b) report on a cross-sectional association between blood lead and SBP among 534 subjects of the same cohort as the critical study of Glenn et al. (2003). Blood lead was measured during the first year of the study; blood pressure was measured annually by a trained technician using a Hawksley random zero sphygmomanometer. Schwartz et al. (2000b) modeled the cross-sectional association between baseline blood lead and blood pressure in two ways: (1) using the data from one visit only; and (2) using data averaged from two annual visits (five measures made over two visits). Covariates included age, BMI, current tobacco use, and current use of anti-hypertensive medications. The paper only presents the results of regression analysis of SBP data from the single visit and reports that the results were not "significantly changed" when using SBP measurements from two visits. A lowess smoothed model of the unadjusted data suggested a non-linear relationship between blood lead and SBP, with little response at lead at concentrations below 5 µg/dL. Both linear and quadratic terms were statistically significant in the covariate adjusted regression model. The authors report a stronger relationship between blood lead and DBP data averaged over two visits, but the results of the analysis with the two-visit averaged SBP were not reported. The study team did not repeat a non-linear analysis in the follow-up longitudinal report for this cohort.

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Figure 24. From Schwartz et al. (2000); figure rotated 90° from original publication; blood lead concentration in μg/dL is on the x-axis. The solid line is a lowess smoothed regression model of the unadjusted data. The dotted line is the adjusted quadratic regression model of the data.

Martin *et al.* (2006) conducted a cross-sectional analysis of the associations between blood lead and blood pressure and hypertension among 964 subjects of the Baltimore Memory Study. The authors used a locally weighted smoothing technique on the residuals of the regression model to identify the presence of a discontinuity in the relationship between blood lead and SBP. The results of this analysis are illustrated in Figure 25. The locally weighted smoothed line of the relationship becomes shallower and less stable at lower blood lead concentrations. The minimum blood lead in the study was not reported, but the analytical limit of detection was 1 µg/dL.



Figure 25. From Martin et al. (2006). Plot of blood lead residuals from adjusted linear regression model and SBP. The dashed line is the adjusted linear regression model. The solid line is a locally weighted smoothing. The smoothed line illustrates the effect of a single influential data at a blood lead concentration of 27.3 µg/dL. The smoothed line also illustrates some attenuation in the slope of the relationship at lower blood lead concentrations.

Pirkle *et al.* (1985) used segmented linear regression analysis to test the cross-sectional relationship between blood lead and SBP of 543 middle-aged Caucasian male subjects of NHANES II (1976-80). While the segmented linear regression analysis indicated that there was not a threshold below which blood lead was not significantly related to blood pressure, the blood lead concentrations amongst his cohort were relatively high: While summary statistics on the study cohort were not reported, it appears from a plot of the study data that only about 10% of subjects had blood lead concentrations less than 10 μ g/dL.

Hense *et al.* (1993) reported on a cross-sectional study of the relationship between blood lead concentrations and blood pressure among subjects of the WHO Monitoring Trends and Determinants in Cardiovascular Disease (MONICA) Ausburg, Germany cohort in 1987 and 1988. There were 3,344 subjects. Males had a median and minimum blood lead concentration of 8.3 μ g/dL and 3.4 μ g/dL, respectively. Females had a median and minimum blood lead concentration of 6.0 μ g/dL and 2.5 μ g/dL, respectively. The authors reported a significant association between blood lead and SBP after adjusting for confounders. The authors also conducted a categorical analysis and binned the data into seven strata. This analysis revealed no evidence of non-linearity or a threshold in the relationship. The lowest bin contained subjects with blood lead concentrations less than 6 μ g/dL.

Nawrot *et al.* (2002) reported the slope of the blood lead-SBP relationship as supralinear (a fixed increase in blood lead per doubling of blood lead). Sixteen of the thirty-one original publications included in the Nawrot *et al.* (2002) meta-analysis reported the shape of the relationship between blood lead and SBP as supralinear (log-linear with either log base e or log base 10). Although not all of these original publications were reviewed, those that were stated that blood lead data were log transformed to satisfy the requirements of normality for linear regression.

Vupputuri *et al.* (2003) reported that, after adjusting for covariates, African-American subjects with blood lead greater than 5 μ g/dL had significantly higher (p < 0.05) SBP than subjects with blood lead concentrations less than 5 μ g/dL. There was no significant difference in the SBP of Caucasian subjects above this blood lead cut-point. The data suggest, at least for African-American females, that the blood lead concentration-response relationship extends below 5 μ g/dL.

Nash *et al.* (2003) reported the mean and SE SBP of the perimenopausal women in their study by quartile blood lead concentrations. The study authors reported a statistically significant linear trend (p < 0.001) of increasing SBP across all quartiles of blood lead, and reported a statistically significant difference (p = 0.03) among the quartile mean SBP values based on an ANOVA. Welch's t-test for unequal variances was used to conduct a step-wise comparison of means for each quartile and found that the only statistically significant difference from the SBP of the 1st quartile was that of the 4th quartile. These data and results are illustrated in Figure 26. While this analysis could be interpreted to suggest a threshold for effect at 3.2 µg/dL (the mean blood lead of the 3rd quartile), this interpretation is dependent on the choice of cut-points (e.g., a different "threshold" would be identified if the data were binned by tertiles) and the power of the study. A visual inspection of the plot does not indicate the presence of a threshold, but again this could be dependent on the choice of bins (e.g., more bins may provide more resolution to identify potential non-linearities).



Figure 26. Mean systolic blood pressure (and SE) by quartile blood lead among 2,165 perimenopausal female subjects of NHANES III as reported by Nash et al. (2003). The p for trend across all quartiles is <0.001. Using Welch's t-test to test the difference between means with unequal variance, only the mean systolic blood pressure of subjects in the 4th quartile of blood lead was significantly different (p < 0.05) than that of subjects in the 1st quartile of blood lead.

Menke *et al.* (2006) did not study SBP as an outcome, but conducted a non-parametric continuous regression analysis of the shape of the blood lead concentration-response relationship for blood lead and all-cause and cause-specific mortality, including cardiovascular mortality. The results of this analysis provide information about the presence of possible population threshold for endpoints that are secondary to increased SBP. Menke *et al.* (2006) used a restricted quadratic spline model to explore the shape of the blood lead concentration-response relationship between blood lead and mortality among 13,946 participants of NHANES III who were followed for up to 12 years for all-cause and cause-specific mortality. The spline model was constructed with knots set at the 10th, 50th and 90th percentiles of the blood lead distribution (1.00, 2.67, and 5.98 µg/dL, respectively). The adjusted spline models from Menke *et al.* (2006) are illustrated in Figure 27.

The spline model for stroke mortality indicates a supralinear blood lead concentrationresponse relationship, while the spline model for myocardial infarction mortality indicates a sinusoidal curve. These data indicate a strong, positive blood lead concentration-response relationship for blood lead and stroke mortality down to at least as low as 1 µg/dL. The shape of the blood lead concentration-response relationship for blood lead and myocardial infarction mortality is not as clear and is attenuated below blood lead concentrations of about 3 µg/dL. While the strength of the blood lead- myocardial infarction mortality may be attenuated over this lower range, this does not preclude the possibility of a remaining blood lead concentration-response relationship between blood lead and SBP. While limited inference can be made from this single study of endpoints which may be—but are not necessarily secondary to the hypertensive effects of lead, it is discussed here because the critical effect of SBP is also representative of other health endpoints that may be adversely effected by lead exposure.



Figure 27. From Menke et al. (2006); Three-knot restricted quadratic spline model of multivariate adjusted relative Hazard Ratios for all-cause and cause-specific mortality by blood lead concentration (µmol/L). A histogram of the distribution of blood lead concentrations among subjects is shown in light grey.

Overall, there is a lack of studies that explicitly tested the shape of the the blood lead-SBP relationship over the lower range of blood lead concentrations. On one hand, several investigators have reported a supralinear relationship. These specifications, however, were not accompanied by rigorous fit testing or other diagnostics to determine the most appropriate functional form of the relationship. Two publications points to a potential attenuation of the relationship at blood lead concentrations less than about 5 µg/dL, but these results are in contrast with data from *in vivo* experiments. In the absence of strong evidence to the contrary, it is assumed that the relationship between blood lead and SBP is linear and that it extends to the lower bounds of the critical study data. However, the certainty of the estimates of the slopes decreases over the lower range of the study data. The mean blood lead concentrations of the subjects of the critical studies were 4.6 µg/dL (Glenn *et al.*, 2003) and 3.4 µg/dL (Vupputuri *et al.*, 2003). Therefore, it is recommend that the estimates of the slopes of the relationship between blood lead concentrations

below about 4 µg/dL be accompanied by explicit statements that qualify the additional uncertainty inherent in the estimates of the slopes over this lower range; the slopes of the blood lead-SBP relationship have not been explicitly examined for non-linearity over the lower range of available data.

There is uncertainty in the functional form of the relationship that best represents (or models) the study data as there is considerable scatter in the study data. The regression models developed by Nash et al. (2003) of the relationship between blood lead and SBP among perimenopausal women from NHANES III achieved R^2 in the range of 0.19 to 0.22; the R^2 s of the regression models developed by Rothenberg et al. (1999) to model the relationship between blood lead and SBP among predominantly Latina women in their third trimester of pregnancy were lower by about a factor of two, with blood lead explaining about 1% of the unexplained variance in SBP. This scatter in the data reduces the ability to identify a single model that best represents the underlying pattern in the data. Neither of the critical studies nor any of the other publications reviewed on the relationship between blood lead and SBP tested alternate functional forms of the modeled blood lead concentration-response relationship. This lack of model testing introduces uncertainty about how well the linear regression models of the critical studies fit the study data relative to other possible functional forms of the blood lead concentration-response relationship. Untested models or differing mathematical constructs of the blood lead concentration-response relationship (e.g., structural equation modeling) may provide alternate estimates of the slope of the blood lead concentration-response relationship. It is impossible to quantitatively estimate the degree to which the lack of alternate model testing introduces model uncertainty, but as evidenced by the quantitative difference in slopes between linear and log-linear models for the relationship between children's blood lead and IQ, model uncertainty can have a very significant effect on the overall quantitative estimate of the slope of the blood lead concentration-response relationship. It is therefore recommended that it be explicitly

emphasized that alternate functional forms of the putative linear blood lead-SBP relationship have not been explored or tested.

Uncertainty in the slope of the model

Both of the critical studies published 95th percent confidence intervals for the slopes of their adjusted linear regression models of the relationship between blood lead and SBP. While these confidence intervals were used to define the recommended upper and lower estimates of the blood lead concentration-response relationship, these confidence intervals only account for the uncertainty about the value of the regression coefficient. They do not account for other sources of uncertainty and variability in the overall estimate of the blood lead concentration-response relationship, such as uncertainty in the functional form of the model. Additionally, the 95th percent confidence intervals for the regression coefficients assume that the independent variable (blood lead) has been measured without error. Random measurement error in the independent variable will be null biasing (e.g., tend to decrease the magnitude of the regression coefficient) and can underestimate the width of the confidence intervals of the regression coefficient (Greene and Ernhart, 1993).

While using the confidence intervals of the slopes of the observed blood lead-SBP relationship to provide a quantitative estimate of the potential uncertainty and variability in the blood lead concentration-response relationship is recommended, caution must be advised about calling them 95th percent confidence intervals because they do not account for all sources of uncertainty. Even within the domain of the uncertainty in the fit of the regression model, they assume that the independent variable was measured without error. Instead, it is recommended that the published confidence intervals on the slopes be used to provide upper and lower estimates of the observed blood lead concentration-response relationship and risk assessors be explicit that the degree of precision of these upper and lower estimates cannot be quantified. The upper and lower estimates of the blood lead concentration-response

relationship represent plausible, but less likely, values for the slope of the relationship. They do not bound the entire range of reported slopes of the blood lead concentration-response relationship, but there are relatively few data supporting estimates of the blood lead concentration-response relationship outside of this range. The upper and lower estimates of the slope of the relationship between blood lead and SBP do not account for the uncertainty in the functional form of the blood lead concentration-response model nor the potential influence of bias and confounding.

Bias and Confounding

Bias and confounding can result in an over-estimation or underestimation of the slope of the blood lead concentration-response relationship. The magnitude of the reported slopes of the relationship between mean population SB and blood lead in adults is undoubtedly influenced by some uncertain degree by confounding. On the other hand, the magnitude of the slope of this relationship is also likely attenuated to some uncertain degree by over-control of potential confounding variables (Bellinger, 2004). For example, many of the studies of blood lead and SBP controlled for the hypertensive effects of smoking, but smoking may also be a surrogate for exposure. Potential sources of bias and confounding are discussed below.

Glenn *et al. (2003)* reported that subjects who had a longer period of follow-up had higher tibia lead concentrations. The relationship between blood lead and SBP was also stronger with longer follow-up periods. This raises the possibility that the subjects with longer follow-up times had higher lead exposure and that these subjects unduly influenced the results (selection bias). The authors, however, repeated their analysis excluding the 5% of subjects with the longest period of follow-up and reported that excluding these subjects affected the adjusted regression coefficient by less than 10%. It does not appear, therefore, that subjects with the longest follow-up period, who also tended to have higher lead exposures, significantly biased the results.

Vupputuri *et al.* (2003) and Glenn *et al.* (2003) both controlled for education level, but no other measures of SES. In addition, neither of these critical studies controlled for noise exposure, which has been associated with SBP and is plausibly associated with increased lead exposure. The absence of control for noise and the limited measure of SES raises the possibility that the magnitude of the reported associations between blood lead and SBP could be influenced by unmeasured or residual confounding. However, since the association between blood lead and SBP has been reported in many study cohorts of varied SES demographics (Nawrot and Staessen, 2006), and since the hypertensive effects of lead exposure have been demonstrated in animal assays and a relevant mechanism of action has also been established, it is unlikely that the association between blood lead and SBP can be wholly attributed to confounding.

The degree to which bias and confounding may have affected the quantitative estimates of the slope of the relationship between adult blood lead and SBP is uncertain. There is no strong evidence that the observed associations from the critical studies were unreasonably affected by selection, measurement or other bias. On balance, there is no compelling evidence to support a qualitative or quantitative adjustment to the estimate of the slope of the relationship between mean population SBP and blood lead in adults to account for the potential influence of bias or confounding.

Summary of Uncertainty & Variability in the Blood Lead-IQ Relationship

In summary, there are multiple sources of variability and uncertainty in the estimate of the slope of the blood lead concentration-SBP relationship. These include:

 Inter-individual variability in susceptibility and the resulting variance in the slope of the blood lead concentration-response relationship

- Uncertainty about what model best describes the underlying relationship
- Uncertainty in the parameters of the model fit to the data of the critical study
- Uncertainty about the influence of bias and confounding

Inter-Study Variance in the Reported Slope of Blood Lead Concentration-BP Relationship

There is evidence to suggest that ethnicity, sex, stress, nutritional status, co-exposure to other xenobiotics, and genetics may modify the blood lead concentration-response relationship between lead and SBP. For example:

- Glenn *et al.* (2001) reported that the slope of the adjusted blood lead-SBP relationship for the 5% of subjects in their study cohort who were homozygous for the variant allele of the ATP1A2 gene was about twelve-fold higher than that for the balance of their cohort. The authors also reported that the variant allele was 1.8 times more prevalent among African-American subjects in their study cohort.
- Vupputuri *et al.* (2003) reported that the slope of the adjusted blood lead-SBP relationship for African-American women was about five-fold higher than that of Caucasian males. Den Hond *et al.* (2002) also reported a higher slope of the adjusted blood lead-SBP relationship for African-American women relative to Caucasian males.
- Peters *et al.* (2007) reported that the slope of the adjusted tibia lead-SBP relationship for subjects of the Normative Aging Study with high levels of self-reported stress was about 2.5 times higher than subjects with low stress.
- Vupputuri *et al.* (2003) also reported that the relationship between blood lead and SBP differed significantly between the sexes; in contrast, however, the meta-analysis of Nawrot *et al.* (2002) reported that the association between blood lead and SBP was similar between the sexes.

• Nash *et al.* (2003) reported that the relationship between blood lead and risk of hypertension was more pronounced in postmenopausal women than premenopausal women.

In the context of the evidence of potential inter-individual variability in the blood lead concentration-response relationship between lead and SBP and the limited representativeness of the study cohort of Glenn *et al.* (2003), it was judged necessary to include an additional critical study to characterize the upper range of the reported variance in observed blood lead concentration-response relationships for blood lead and SBP. The adjusted regression coefficient for the relationship between blood lead and SBP among African-American female subjects of the Vupputuri *et al.* (2003) study represents the steepest slope of the blood lead-SBP relationship for a potentially susceptible sub-population that has been identified as a susceptible sub-population in multiple studies. The slope from Vupputuri *et al.* (2003) accounts for some, but not all, of the reported variance in slopes of the blood lead concentration-SBP relationship.

Uncertainty in the Extent and Shape of the Blood Lead-SBP Relationship

A literature search revealed few publications that explicitly tested the shape of the blood lead concentration-response relationship between blood lead and SBP or attempted to identify a threshold for the hypertensive effects of lead. In the absence of evidence to the contrary, it is recommended that the slope of the blood lead concentration-response relationship between blood lead and SBP be assumed to be linear. However, the certainty of the estimates of the slopes decreases over the lower range of the study data. The mean blood lead concentrations of the subjects of the critical studies were 4.6 μ g/dL (Glenn *et al.*, 2003) and 3.4 μ g/dL(Vupputuri *et al.*, 2003). Therefore, it is further recommended that the estimates of the slope of the relationship between blood lead and SBP for blood lead concentrations below about 4 μ g/dL be accompanied by explicit statements that qualify the additional

uncertainty inherent in the estimates of the slopes over this lower range; the slopes of the blood lead-SBP relationship have not been explicitly examined for non-linearity over the lower range of available data.

Bias and Confounding

The observed relationship between blood level and SBP is unlikely to be entirely attributable to confounding: Lead exposure causes hypertensive effects in experimental animals, there is strong evidence of biological plausibility, and the association between blood lead concentrations and SBP has been observed in multiple cohorts of divergent ethnic, social and economic demographics. While the magnitude of the slope of the recommended relationship between mean population SBP and blood lead in adults is undoubtedly influenced by some unknown degree by confounding, it is also likely attenuated by overcontrol. There is no evidence that the observed association from the critical studies were unreasonably affected by selection, measurement or other bias.

Accounting for Variability and Uncertainty

The 95th percent confidence intervals on the adjusted log-linear regression coefficient from the critical study are recommended to partially quantify the potential influence of these sources of variability and uncertainty. However, the confidence intervals are derived only based on the uncertainty in the value of the model parameter (the regression coefficient). They do not quantitatively account for other sources of uncertainty and variability in the overall estimate of the blood lead concentration-response relationship. While the upper and lower estimates of the blood lead concentration-response relationship represent plausible, but less likely, values for the slope of the relationship, they do not bound the entire range of reported slopes. There are relatively few data supporting estimates of the blood lead concentration-response.

Recommended Slope and Sample Calculations

The parameters for the upper and lower estimates of the slope of the relationship between population mean SBP and blood lead in adults are presented in Table 19 below. These slopes are illustrated in Figure 28.

Table 19. Summary of recommended slopes of the relationship between blood lead in adults and population meansystolic blood pressure.

Endpoint	Critical Study	Slope	
Lower estimate, Caucasian males	Glenn <i>et al.</i> (2003)	0.05 mmHg per µg/dL	
Best estimate, Caucasian males	Glenn <i>et al.</i> (2003)	0.25 mmHg per µg/dL	
Best estimate, susceptible sub- populations	Vupputuri <i>et al</i> . (2003)	0.47 mmHg per µg/dL	
Upper estimate, susceptible sub- populations	Vupputuri <i>et al</i> . (2003)	0.80 mmHg per µg/dL	



Figure 28. Recommended slopes of the relationship between adult blood lead and population mean systolic blood pressure. The dark solid lines are the recommended best estimate slopes for Caucasian males and susceptible sub-populations. The light solid lines are the lower and upper estimates of the slopes. The progressive shading indicates increasing uncertainty in the estimates of the slopes. The slopes have not been tested for non-linearity; 4 µg/dL is the approximate mid-point of the data for the critical studies.

The longitudinal study of the relationship between baseline blood lead and change in SBP over time among former lead workers by Glenn *et al.* (2003) was selected as the critical study upon which to base a quantitative estimate of the slope of the relationship between blood lead and SBP. The slope and 95th percent confidence intervals of the adjusted linear regression coefficient reported by Glenn *et al.* (2003) is 0.25 (0.05 to 0.45) mmHg per year per μ g/dL. To meet the requirements of risk assessors and to make the results comparable with other epidemiological studies, it was assumed that the relationship was cross-sectional (static) and simplified the slope to 0.25 (0.05 to 0.45) mmHg per μ g/dL concurrent blood lead. It is recommended that the slope of the adjusted regression model from the critical study of Glenn *et al.* (2003) (0.25 mmHg per μ g/dL) be used to represent the best estimate slope of the relationship between adult blood lead and SBP among Caucasian males. Furthermore, it is recommended that the lower 95th percent confidence interval on the slope of the adjusted regression model from the critical study of Glenn *et al.* (2003) (0.05 mmHg per μ g/dL) be

used to provide a lower estimate of the slope of the relationship between adult blood lead and SBP among Caucasian males and other potentially non-susceptible sub-populations.

Ethnicity, sex, stress, nutritional status, co-exposure to other xenobiotics, and genetics may modify the blood lead concentration-response relationship between lead and SBP. There is suggestive evidence that the slope of the blood lead concentration-response relationship may be steeper by up to a factor of about ten for susceptible sub-populations. In light of the observed variability in the slope of the relationship between blood lead and SBP, a slope is also recommended for susceptible sub-populations. The magnitude of this slope is based on the results of a cross-sectional linear regression analysis of the relationship between blood lead and SBP among African-American female participants of NHANES III (Vupputuri *et al.*, 2003). The slope is intended to be representative of all potentially susceptible sub-populations and not exclusively African-American females. The recommended slope for susceptible sub-populations was based on a critical study of African-American females because evidence that identifies African-American females as a susceptible sub-population has been replicated in independent studies. It must be emphasized, however, that there are un-replicated data that suggest a steeper blood lead concentration-response relationship for some susceptible sub-populations.

Vupputuri *et al.* (2003) do not report a regression coefficient and SE from their analysis, but instead report that a one standard deviation increase in blood lead (3.3 μ g/dL) was associated with an adjusted increase (95th percent confidence intervals) in SBP of 1.55 (0.47 to 2.64) mmHg in African American women. The calculated equivalent adjusted regression coefficient and 95th percent confidence intervals are: 0.47 (0.14 to 0.80) mmHg per μ g/dL. It is recommended that the slope of the adjusted regression model from the critical study of Vupputuri *et al.* (2003) (0.47 mmHg per μ g/dL) be used to represent the best estimate of the slope of the relationship between adult blood lead and SBP among potentially susceptible sub-populations. Furthermore, it is recommended that the upper 95th percent confidence

interval on the slope of the adjusted regression model from the critical study of Vupputuri *et al.* (2003) (0.80 mmHg per μ g/dL) be used to provide an upper estimate of the slope of the relationship between adult blood lead and SBP among susceptible sub-populations.

In addition to two slopes to represent the potential variability in blood lead concentrationresponse amongst sub-populations, upper and lower estimates of the blood lead concentration-response slopes are also recommended to further characterize the potential variability and uncertainty in the estimate of the slope. The upper and lower estimates of the slopes are derived from the upper and lower 95th percent confidence intervals of the adjusted linear regression coefficients from the critical studies. While these upper and lower estimates are based on, and to some degree, account for, the uncertainty in the slope of the adjusted regression coefficient, they are also based on the assumption that blood lead has been measured without error and do not explicitly account for additional sources of uncertainty (e.g., model uncertainty or the uncertain influence of bias and confounding). Therefore, it is not recommended that these upper and lower estimates of the slope of the relationship be qualified by inferring a degree of precision in the estimates (such as 95th percent confidence intervals). Rather, the upper and lower estimates of the blood lead concentration-response relationship represent plausible, but less likely, values for the slope of the relationship. They do not bound the entire range of reported slopes of the blood lead concentration-response relationship, but there are relatively few data supporting estimates of the blood lead concentration-response relationship outside of this range. The upper and lower estimates of the slope of the relationship between blood lead and SBP do not account for all sources of variability and uncertainty in the estimate of the slope of the relationship between blood lead and SBP.

It is emphasized that:

- The upper and lower estimates of the blood lead concentration-response relationship represent plausible, but less probable, values for the slope of the relationship.
- The upper and lower estimates of the blood lead concentration-response relationship do not bound the entire range of reported slopes of the blood lead concentration-response relationship.
- The slope of the relationship has not been tested for nonlinearity over the lower range of study data and estimates of the slope of the relationship are increasingly uncertain below blood lead concentrations of about 4 µg/dL.
- Estimates of the blood lead-SBP relationship below 1 µg/dL are based on extrapolation of the relationship beyond the range of study data and the uncertainty in the estimate of the slope of the relationship further increases with increasing magnitude of extrapolation.
- To-date, alternate functional forms of the putative linear blood lead-SBP relationship have not been explored or tested.
- The degree to which bias and confounding may have affected the quantitative estimates of the slope of the relationship between adult blood lead and SBP is uncertain.

Equation 3 is used for calculating estimates of change in mean population SBP (Δ SBP) associated with incremental changes in adult blood lead.

Equation 3

 $\Delta SBP = \beta_1(x_2 - x_1)$

Where β_1 = the adjusted linear regression coeficient from the critical study

 X_1 = the lower blood lead concentraiton

 X_2 = the higher blood lead concentration

Х ₁ (µg/dL)	Х₂ (µg/dL)	Delta blood Pb (μg/dL)	Lower estimate of delta SBP ¹	Best estimate of delta SBP among Caucasian males ¹	Best estimate of delta SBP among susceptible sub- populations ¹	Upper estimate of delta SBP ¹
4.0	5.0	1.0	0.1	0.3	0.5	0.8
3.0	5.0	2.0	0.1	0.5	0.9	1.6
1.0	4.0	3.0	0.2	0.8	1.4	2.4
1.0	5.0	4.0	0.2	1	1.9	3.2

Table 20. Examples of application of the recommended slopes of the relationship between blood lead in adults and population mean SBP to hypothetical changes in blood lead.

1. Change in population mean SBP is estimated to a tenth of an mmHg, because population SBP is reported in the critical studies with this degree of precision.

Table 20 presents the results of applying the recommended slopes to a series of hypothetical changes in blood lead concentrations in adults. A change in blood lead in adults from $1.0 \ \mu g/dL$ to $4.0 \ \mu g/dL$, which is estimated to be approximately equivalent to the 25^{th} to 95^{th} percentile of blood lead concentrations in Canadian adults, is associated with an estimated increase in mean SBP among adults of approximately 0.2 to 2.4 mmHg, with a best estimate of an increase of 0.8 mmHg among Caucasian males and an increase of 1.4 mmHg among susceptible sub-populations. The potential range of increase in SBP may be larger because the estimates provided do not account for all potential sources of uncertainty and variability in the blood lead concentration-response relationship.

5.5 SUMMARY OF ESTIMATED BLOOD LEAD CONCENTRATION-RESPONSE SLOPES

The objective of Section 5 was to recommend quantitative estimates of the blood lead concentration-response relationships for IQ in children and SBP in adults.

The emphasis in this chapter is on quantifying blood lead concentration-response relationships over the range of contemporary blood lead concentrations among Canadians. Estimates of the blood lead concentration-response relationships for the primary endpoints are required so that risk assessors can estimate the change in population health outcomes associated with incremental changes in environmental lead exposures. Because blood lead concentration-response data are available from epidemiological studies where exposures are within or very close to the range of contemporary blood lead concentrations among Canadians, the estimated blood lead concentration-response relationships can be made with a relatively high degree of scientific certainty.

This section of the report:

Presents an estimation of the current distribution of blood lead concentrations in environmentally exposed Canadians. Preliminary results from the nationally representative Canadian Health Measures Survey (CHMS) report that the 25^{th} and 95^{th} percentiles of blood lead concentrations among Canadians 20 to 69 years old in 2007 and 2008 were approximately 1 and 4 µg/dL, respectively. The 25^{th} and 95^{th} percentiles of blood lead concentrations in children six to 19 years of age are 0.6 and 1.6 µg/dL, respectfully. There are no nationally representative data available for Canadian children less than six years of age. By several lines of reasoning, it is estimated that the 25^{th} and 95^{th} percentiles of blood lead concentrations among Canadian children less than six years of age. By several lines of reasoning, it is estimated that the 25^{th} and 95^{th} percentiles of blood lead concentrations among Canadian children less than six years of age. By several lines of reasoning, it is estimated that the 25th and 95th percentiles of blood lead concentrations among Canadian children less than six years of age. By several lines of reasoning, it is estimated that the 25th and 95th percentiles of blood lead concentrations among Canadian children less than six years old are 1 and 4-6 µg/dL, respectively.

Identifies a critical study that best represents the slope of the relationship between blood lead concentrations and IQ decrements in environmentally exposed Canadian children. An international pooled analysis of seven longitudinal studies by Lanphear *et al.* (2005) was selected as the critical study that best represents the expected blood lead concentration-IQ relationship among environmentally exposed Canadian children. *Critically reviews the sources of uncertainty and variability in the recommended slope of the blood lead concentration-IQ relationship.* Most studies that have examined the shape and the extent of the blood lead concentration-IQ relationship provide evidence that the slope of the relationship is steeper at lower blood lead concentrations and multiple studies provide evidence that this relationship extends at least as low as 1 μ g/dL. While the weight of evidence supports this conclusion, the available studies are not unanimous in this conclusion and there are other important sources of uncertainty and variability in the estimated slope of the blood lead concentration-IQ relationship. The 95th percent confidence intervals on the covariate adjusted regression coefficient from the critical study quantify some, but not all, of the uncertainty and variability in the overall estimate of the slope of the relationship.

Applies the recommended blood lead concentration-IQ slope to estimate the change in population mean IQ associated with an increase in children's blood lead concentrations from 1 to 4 μ g/dL (approximately the 25th and 95th percentile blood lead concentrations in Canadian children less than 6 years old). An increase in children's blood lead concentrations from 1 to 4 μ g/dL is associated with an estimated decrement in population mean IQ of 2.3 to 5.2 IQ points, with a best estimate of 3.7 IQ points. However, the potential range of IQ decrements may actually be larger because the estimates provided do not account for all potential sources of uncertainty and variability in the blood lead concentration-IQ relationship.

Identifies a critical study that best represents the expected slope of the relationship between blood lead concentrations and systolic blood pressure in environmentally exposed Canadian adults. A longitudinal study of formerly occupationally exposed organolead workers by Glenn *et al.* (2003) was selected as the critical study that best represents the expected blood lead concentration-SBP relationship among environmentally exposed Caucasian males. Because of the limited diversity of subjects in the study by Glenn *et al.* (2003), the variance in reported slopes of the blood lead concentration-SBP relationship, and the evidence of significant variance in susceptibility amongst sub-populations, a second critical study was identified to represent the potential blood lead concentration-SBP response among susceptible sub-populations. A cross-sectional study of blood lead concentrations and SBP among adult female African-American subjects of the US National Health and Nutrition Examination Study by Vupputuri *et al.* (2003) was selected as the critical study that best represents the expected blood lead concentration-SBP relationship among susceptible sub-populations, including but not limited to African-American females.

Critically reviews the sources of uncertainty and variability in the recommended slope of the blood lead concentration-SBP relationship. Few analyses of the extent and shape of the blood lead concentration-SBP relationship are available in the literature. In the absence of compelling evidence to the contrary, it is recommended that the shape of the relationship be assumed linear. Because the relationship has not been extensively tested for non-linearity, there is increasing uncertainty in the strength and extent of the relationship below blood lead concentrations of about 4 μ g/dL. There are other important sources of uncertainty and variability in the estimated slope of the blood lead concentration-SBP relationship. The 95th percent confidence intervals on the covariate adjusted regression coefficients from the critical studies quantify some, but not all of the uncertainty and variability in the slope of the relationship.

Applies the recommended blood lead concentration-SBP slope to estimate the change in population mean SBP associated with an increase in adult blood lead concentrations from 1 to 4 μ g/dL (approximately the 25th and 95th percentile blood lead concentrations in Canadian adults). An increase in adult blood lead concentrations from 1 to 4 μ g/dL is associated with an estimated increase in population mean SBP of approximately 0.2 to 2.4 mmHg, with a best estimate of 0.8 mmHg among Caucasian males
and 1.4 mmHg among susceptible sub-populations, such as African-American females. The potential range of increase in SBP may be larger because the estimates provided do not account for all potential sources of uncertainty and variability in the blood lead concentration-SBP relationship.

Slopes of the blood lead concentration-response relationship for primary endpoints are provided so that risk assessors can calculate estimates in changes in health endpoints associated with changes in blood lead concentrations. It is emphasized, however, that:

- There is evidence that lead exposure can adversely affect many health endpoints, not just those for which quantitative estimates of the blood lead concentration-response relationship are provided (IQ and SBP).
- The recommended blood lead concentration-response relationships describe the expected change in the population mean of the health endpoint associated with changing blood lead concentrations. Health effects at an individual level cannot be estimated from the blood lead concentration-response relationships recommended herein.
- In applying the recommended slopes, the uncertainty in the estimates of potential health effects associated with a change in blood lead concentration should be acknowledged, presented transparently and, where possible, quantified.

SECTION 6 • RISK-SPECIFIC BLOOD LEAD CONCENTRATIONS

6.1 INTRODUCTION

Section 5 of the report provides estimates of the slope of the relationship between blood lead concentrations and IQ and systolic blood pressure (SBP). These estimates are based on epidemiological studies of the blood lead concentration-response relationships over ranges of lead exposure that are close to or within the range of current environmental lead exposures for most Canadians. The estimated slopes are provided so that risk assessors can calculate the incremental changes in population health endpoints associated with incremental changes in blood lead concentrations that are within the range of the critical study data. In cases, however, where risk assessors are required to evaluate the potential health risks associated with exposures that are less than current environmental lead exposures, estimates of the of the exposure-response relationships will necessarily be based on an extrapolation of the relationship below the range of the critical study data.

The purpose of this section is to provide risk-specific blood lead concentrations for population effects on IQ and SBP. This requires that the estimates of the exposure-response relationships for IQ and SBP be extrapolated below the range of study data. The exposure-response relationships are extrapolated to zero exposure so that the slopes can be used both to estimate incremental risks associated with lead exposures that are less than the critical study data, and to derive estimates of absolute risk or risk-specific blood lead concentrations.

The extrapolated exposure-response relationships and the risk-specific blood lead concentrations are presented in a separate section of the report for two reasons: (1) the extrapolated exposure-response relationships are based on scientific judgement and

reasoning, rather than exclusively on data; and (2) the derivation of risk-specific blood lead concentrations necessitates the definition of an associated "target" risk. Because the target risks are expressed in absolute terms, the risk-specific metrics are also based on an exposure-response relationship extrapolated to zero exposure. Because of the increased dependence on scientific judgement and reasoning as opposed to direct empirical evidence, it was felt to be more transparent to present the derivation of extrapolated exposure-response relationships and the risk-specific blood lead concentrations in a separate section of the report.

This section:

Presents a rationale for deriving a probabilistic risk-specific blood lead concentrations rather than attempting to derive a "threshold" toxicological reference value (TRV) for lead. TRVs for non-carcinogens have traditionally been defined as a "threshold" below which there is no risk of adverse effects. There are, however, fundamental and operational issues with this traditional approach. Fundamentally, the threshold TRV approach does not allow exposure risks to be characterized quantitatively. Non-cancer risks have been traditionally characterized qualitatively as either "acceptable" or "unacceptable". Operationally, existing data do not provide strong evidence of a threshold for the critical effects of lead and there is rationale to suggest that these effects may not have a threshold when viewed from a population perspective. Therefore, the TRVs for lead derived in this report are blood lead concentrations associated with some *a priori* defined probability of effect. This approach was taken because the currently available data do not identify, with confidence, a blood lead concentration that is without risk of adverse population effects.

Employs a benchmark dose (BMD) approach to calculate benchmark blood lead concentrations (BMCs). The BMD approach uses the blood lead concentration-response slopes from the published critical studies to calculate the blood lead concentrations associated with a defined level of response—the benchmark response (BMR).

- For childhood IQ, the BMCs were calculated for a BMR of a 1 point decrement in population mean IQ. On a population basis, a mean decrement in IQ of 1 point is associated with an added risk of mild mental retardation (MMR) of approximately 1 in 250 children (385 in 100,000).
- The best estimate BMC associated with a 1 point decrement in mean IQ is 0.3 µg/dL, with a lower estimate BMC of 0.2 µg/dL and an upper estimate BMC of 1.4 µg/dL. The lower and upper estimates of the BMC reflect some, but not all, of the variability and uncertainty in the estimate of the slope of the blood lead concentration-IQ relationship.
- For SBP, the BMCs were calculated for a BMR of an increase in the population mean SBP of 1.3 mmHg. On a population basis, a mean increase in SBP of 1.3 mmHg is associated with a 1 in 20 (5,421 per 100,000) added risk of hypertension and a 1 in 2,000 (50 per 100,000) incremental risk of coronary heart disease mortality in adults.
- The best estimate of the BMC for a 1.3 mmHg increase in mean SBP is 5 µg/dL for Caucasian males and 2.7 µg/dL for susceptible sub-populations. The lower estimate BMC is 1.6 µg/dL and the upper estimate BMC is 25 µg/dL. The lower and upper estimates of the BMC reflect some, but not all, of the variability and uncertainty in the estimate of the slope of the blood lead concentration-SBP relationship.

Presents estimates of a blood lead distributions around the BMCs to derive riskspecific blood lead concentrations. A risk-specific blood lead concentration is the geometric mean blood lead concentration associated with a specified probability of population health effects. Data from national blood lead biomonitoring studies indicate that the current ratio of 95th percentile to geometric mean blood lead concentrations in the population is about three. Therefore, the risk-specific blood lead concentrations were derived by dividing the BMCs by a factor of three.

- The best estimate of the children's blood lead concentration where the mean leadassociated IQ decrement will be no more than 1 IQ point in 95% of the population is 0.1 µg/dL with a lower and upper estimate of 0.1 to 0.5 µg/dL, respectively. At this geometric mean blood lead concentration, 5% the population would have an average IQ loss of 1 point or greater.
- The best estimate of the adult blood lead concentration where the mean leadassociated increase in SBP will be no more than 1.3 mmHg in 95% of the population is 1.7 µg/dL for Caucasian males and 0.9 µg/dL for susceptible sub-populations. The lower and upper estimates are 0.5 and 6.3 µg/dL, respectively. At this geometric mean blood lead concentration, 5% of the population would have an average increase in SBP of 1.3 mmHg or greater.

The risk-specific blood lead concentrations derived in this report are intended to provide risk managers with a point of reference for making decisions about primary prevention of population health effects from lead exposure. The risk-specific blood lead concentrations are not intended to represent a threshold of effects, nor are they intended to represent an "acceptable" or "tolerable" risk. They simply quantify the blood lead concentrations associated with a specified level of population risk. The methods presented in this report may be used by risk assessors to calculate risk-specific blood lead concentrations associated with higher or lower population risk levels.

6.2 BACKGROUND

6.2.1 Toxicological Reference Values

Toxicological reference values (TRVs) are benchmarks used to guide primary prevention of chemical exposures and include metrics of *absolute* risk (reference doses, tolerable daily intakes, acceptable daily intakes, etc.) and metrics of potency so that *incremental* risks can be calculated. Historically TRVs for non-carcinogens were expressed as an absolute metric because it was assumed that they were based on a threshold of effects below which exposures presented no risk of harm. Alternatively, the assumption for carcinogens, except those for which there was evidence of a threshold mode of action, was that there was a health risk associated with any level of exposure and, thus, TRVs for carcinogens are an expression of the chemical's cancer potency.

The conceptual distinction between carcinogens as non-threshold substances and noncarcinogens as threshold substances, however, has been criticized. Several authors, including a US National Research Council (US NRC) Committee on Improving Risk Analysis Approaches used by the US EPA, have recommended moving away from this dichotomy in approaches in favour of harmonizing methods of dose-response characterization and risk characterization (Crawford and Wilson, 1996; Crump *et al.*, 1997; Gaylor *et al.*, 1999; Gaylor and Kodell, 2002; Hattis *et al.*, 2002; Castorina and Woodruff, 2003; US National Research Council, 2008). Both the US NRC (2008) Committee on Improving Risk Analysis Approaches used by the US EPA and the US EPA Science Advisory Board (2002) have recommended developing quantitative and probabilistic methods for characterizing risk for non-cancer endpoints.

The primary objections to the historical approach for non-carcinogens are that:

- The assertion that there is no health risk below a given dose cannot be conclusively supported in science because one cannot "prove" a negative (i.e., it is impossible to conclusively demonstrate an absence of effect).
- Non-carcinogens are assumed to have a non-linear dose-response and this determination is made without consideration of other risk factors, such as chemical exposures or disease processes, which may modify the dose-response relationship at low exposures.
- Exposure risks for non-carcinogens are only qualified as either acceptable or unacceptable: Non-cancer TRVs and hazard quotients (or hazard indices) do not quantify the associated likelihood or magnitude of harm or risk at various exposures. Therefore, they provide very limited information to support transparent risk communication and are inadequate for quantitative benefit-cost analysis and comparative risk analysis. Non-cancer TRVs provide no indication of the expected risk reduction associated with reducing exposures.
- The use of uncertainty factors (UFs) engenders misunderstandings that these values are "safety" factors and that the use of UFs produces threshold TRVs that are highly or overly conservative.
- The current dichotomy of approaches between carcinogens and non-carcinogens creates inequity and inconsistency in the assessment and management of chemical exposure risks; risks from non-carcinogens tend to be under-represented. Non-cancer endpoints tend to be afforded little weight in benefit-cost analysis because of their associated qualitative risk characterization.

In response to these objections, the US NRC (2008) Committee on Improving Risk Analysis Approaches used by the US EPA recommended a unified approach to dose-response assessment. Under the proposed unified approach, the shape of the population doseresponse curve would be determined through a formal and systematic assessment of background disease processes and exposures and inter-individual variability in susceptibility. In the absence of evidence to the contrary, both carcinogens and non-carcinogens are assumed to have low-dose linear population dose-response curves. The unified approach would further re-define TRVs for non-carcinogens as probabilistic risk-specific doses (analogous to the concept of risk-specific dose as it is currently applied in risk assessment of carcinogens). This alternate framework for risk assessment provides the advantage that it permits quantitative estimates of benefits associated with alternate risk management options, is more faithful to the underlying science, is consistent among chemicals, and does not impose artificial distinctions among health endpoints.

Several referees of a draft version of this report recommended that Health Canada follow the recommendations of the US NRC (2008) in finalizing this report and deriving recommended TRVs for lead. The US NRC (2008) report and related literature were reviewed and it was judged that, both conceptually and practically, it would be preferable to define the dose-response relationships and associated risk-specific doses for key endpoints rather than attempt to define, as would have been done under the historical paradigm, a threshold of exposure that is expected to be without risk of health effects. The conceptual disadvantages of a threshold TRV were discussed above. These, in the context of the practical reality that there is little evidence of an identifiable population threshold for some health effects of lead, present compelling rationale to adopt the approach recommended by the US NRC (2008).

6.3 METHODS

The following sections present the methods used to calculate the risk-specific blood lead concentrations. The same methods may be used to calculate blood lead concentrations associated with higher or lower levels of risk.

The general steps used to calculate risk-specific blood lead concentrations are:

1. Define a benchmark level of risk (or response).

- 2. Define the mathematical model of the blood lead concentration-response relationship.
- 3. Calculate the benchmark blood lead concentration associated with the benchmark risk
- 4. Assume a population distribution for blood lead concentrations.
- 5. Calculate the geometric mean blood lead of distribution, where the 95th percentile of the distribution is equivalent to the benchmark blood lead concentration.

The last two steps are included to ensure that a population with a geometric mean blood lead concentration equivalent to the risk-specific blood lead concentration has a 95% probability of risk equal to or less than the benchmark level of risk.

While some of the language and concepts from the benchmark dose (BMD) methods of deriving a TRV have been used, it is important to note that the traditionally applied BMD methods were completely followed. Principally this is because a dose-response model was not fit, de nova, to the critical study data. While this would have been desirable, the original study data could not be obtained, and the critical studies all included published doseresponse models. Additionally, in the case of IQ, the fit of the published dose-response model was well justified in both the critical study and in an independent published analysis of the same data. A second departure from the commonly applied BMD method is a benchmark response (BMR) relative to background or control was not calculated. This is because the epidemiological design of the critical studies did not measure this parameter. It was assumed that at a blood lead concentration of 0 µg/dL there will be no effect on IQ or SBP. A third difference between the methods used in this report and the traditional BMD approach is that the lower confidence limit on the benchmark dose (BMDL) is normally used as a point of departure (POD) and the POD is divided by uncertainty factors to produce a "threshold" TRV. Because the objective of this report was to define the blood lead concentration associated with a specified level of risk, the confidence intervals (both upper and lower) on the BMD were used to help quantify the uncertainty in the estimate. No reference is made to the intervals as precisely defined confidence intervals (e.g., 95th percent confidence intervals),

because the statistical methods by which they were derived do not quantitatively account for all sources of variability and uncertainty in the estimate. The BMD was not divided by any uncertainty factors because the objective of this report was not to identify a point of departure (POD) for determining a threshold TRV, but simply to identify a specific level of response based on a blood lead concentration-response relationship. Finally, because the exposure metric in the critical studies selected is blood lead concentration, the benchmark exposures have been defined as benchmark concentrations (BMCs), rather than BMDs.

It is also noted that, in selecting a risk level to calculate a risk-specific blood lead concentration, there are two competing objectives. On one hand, it is desirable to base the calculations on a level of response (risk) that is associated with blood lead concentrations that were within the range of critical study data. On the other hand, the risk-specific blood lead concentrations must be meaningful points of reference for risk managers faced with making decisions about possibly reducing contemporary environmental lead exposure levels; therefore, the associated blood lead concentrations are necessarily in the low range or below current blood lead concentrations and the range of study data. The blood lead concentrations associated with a 1.3 mmHg increase in mean SBP are within the range of the critical studies used to define the slopes of the blood lead-SBP relationship. The blood lead concentrations associated with a 1 point decrement in mean IQ are within a factor of three of the minimum data of the critical study used to define the slope of the blood lead-IQ relationship. While the extrapolation increases the uncertainty associated with these estimates, this uncertainty is mitigated by the evidence that the slope of the blood lead-IQ relationship is steeper, rather than shallower, over the lower ranges of available data. Risk assessors who use the methods presented here for calculating risk-specific blood lead concentrations associated with lower levels of risk (i.e., lower benchmark responses) should be mindful of the increasing uncertainty in the shape and extent of the blood lead concentration-response relationships as one extrapolates the relationship below the range of study data.

6.3.1 Defining a Benchmark Response

The objective of this section is to calculate the blood lead concentration associated with a defined change in the critical endpoint (SBP or IQ). This level of response—the benchmark response (BMR)-was defined a priori. For continuous endpoints, such as IQ and SBP, there are several different possible ways to express a BMR. These options are described and reviewed by Filipsson et al. (2003) and they include point, absolute, relative, extra, and scaled effect. While Crump (Crump, 1984; Crump, 1995) originally defined BMD as the dose that corresponds to a BMR relative to control or unexposed subjects, due to the ubiquity of lead in the environment there are no controls or unexposed subjects in the critical studies used and, therefore, this parameter is unknown. Instead, the BMRs were defined as (1) an absolute increase in SBP of 1.3 mmHg (approximately equivalent to 1% of the average SBP among Canadian adults); and (2) an absolute decrement in IQ of 1 point (by definition equivalent to 1% of the national average IQ). It was assumed that there is no lead effect at a blood lead concentration of 0 µg/dL (i.e., the intercepts of the blood lead concentrationresponse models presented in this report were assumed to be zero). Therefore, the BMR for SBP was defined as 1.3 mmHg and the BMR for IQ was defined as -1 IQ point. By this method, the only parameter from the mathematical blood lead concentration-response relationship that is required for calculation of the benchmark concentration (BMC) is the slope of the model. In all cases, published adjusted regression coefficients from the critical studies were used as the model slopes for calculating BMCs. The population health significance of BMRs of this magnitude is discussed in detail below. The BMD method also requires that the blood lead concentration-response relationship be extrapolated to a blood lead concentration of 0 μ g/dL. Therefore a low-dose linear dose-response for both IQ and SBP was assumed; the justification for this assumption is provided below.

6.3.2 Population Risks Associated with a 1% Response

The population risks associated with the risk-specific blood lead concentrations calculated in this report are:

- The average blood lead concentration in children that is associated with an average IQ loss of 1 point or less in 95% of the population. At this blood lead concentration, 5% of the population would have an average IQ loss of 1 point or greater.
- The average blood lead concentration in adults that is associated with an average increase in SBP of 1.3 mmHg or less in 95% of the population. At this blood lead concentration, 5% of the population would have an average increase in SBP of 1.3 mmHg or greater. An increase in SBP of 1.3 mmHg is approximately equivalent to a 1% increase in the average SBP among Canadian men and women 35 to 64 years old.

The risk-specific blood lead concentrations are not intended to represent a threshold of effects, nor are they intended to represent an "acceptable" or "tolerable" risk. They simply quantify the blood lead concentrations associated with a specified level of population risk. The following discussion is provided to assist risk assessors and risk managers in understanding the specified level of population risk associated with the risk-specific blood lead concentrations. The methods presented in this report may be used by risk assessors to calculate risk-specific blood lead concentrations associated with higher or lower population risk levels.

The risk-specific blood lead concentrations derived in this report are intended to provide risk managers with a point of reference for primary prevention of population health effects from lead exposure. In the context of primary prevention, such as determining allowable levels of lead in the environment, health risks are considered from a population perspective. This raises Rose's (1981) concept of the "prevention paradox", where a preventive measure that brings a significant benefit to the population may appear to offer little benefit to each participating individual. Because both the costs and benefits of population risk reduction or prevention are amortized over the entire population, health agencies may take action to

reduce health risks that are so small as to be essentially meaningless on an individual basis (e.g., a 1 mmHg increase in mean systolic blood pressure). However, changing the population mean of a risk factor by a small margin may result in a significant net health improvement at the population level, particularly if the risk factor is for a relatively common and costly health outcome. The following discussion illustrates the population health implications of a 1% decrement in children's IQ and a 1% increase in adult SBP:

A 1% change in population IQ is equal to a change of 1 IQ point (IQ tests are normalized to a mean of 100 points). The mean SBP among Canadian males 35 to 74 years old (data from 1986-92) was 132.2 mmHg (Canadian Heart Health Database, 1992). The mean SBP among Canadian females 35 to 74 years old (data from 1986-92) was 127.4 mmHg (Canadian Heart Health Database, 1992). The average blood pressure among all Canadian adults was, therefore, estimated to be 130 mmHg. A 1% BMR in population SBP is equivalent to 1.3 mmHg.

At first consideration, a 1% change in the average IQ or SBP in a population may seem insignificantly small as both are within the range of intra-individual variation and individual measurement error. However, it is important to remember that this magnitude of change in these endpoints is in the population average, not on an individual basis and the mean differences of this magnitude (1% difference in IQ or SBP) between populations can be reliably measured. To understand the significance of these incremental changes in the critical endpoints, the related population level health effects must be examined.

Several lines of inquiry may be examined to understand the significance of a small shift in the population distribution of IQ or SBP. As mentioned in previous sections, both of these endpoints are risk factors for other health outcomes such as mild mental retardation (MMR) and cardiovascular mortality. Using methods described below, one can predict how changes in the population distribution of IQ and SBP can affect the population risk for related adverse

outcomes, such as MMR and clinical cardiovascular morbidity and mortality, respectively. Because the economic costs of these more definitive related outcomes have been quantified, it is also possible to estimate the societal costs associated with small shifts in the population distribution of IQ or SBP.

There is a strong association between IQ and measures of socioeconomic status, such as school grades, years of education, job success, social status and income. These outcomes are, in turn, important social determinants of health status and lifespan. Herrnstein and Murray (1994) estimated that a 3% increase in population IQ would be associated with an average 20% reduction in social outcomes such as out-of-wedlock birth, low birth weight, welfare dependence, poverty rate, incarceration rates, and high school drop-out rates. Schwartz (1994b) calculated that a 1 point change in population mean IQ was associated with a 4.5% change in the probability of graduating from high school.

Nevin *et al.* (2000) estimated that each IQ point increase raises the worker productivity and translates into an increase in US citizen lifetime earnings of \$16,809 (2005 dollars). If it is assumed that the non-discounted value of lifetime earnings of a Canadian is equivalent, and that 342,176 babies were born in Canada in 2005, the national cost associated with a 1 point decrement in mean population IQ can be crudely estimated at about six billion dollars in lost lifetime earnings per birth year cohort.

Normal distribution theory may also be used to estimate the additional or extra risk of the probability of a dichotomous adverse outcome amongst an exposed population (Crump, 1995). The approach assumes that IQ and SBP are normally distributed and have a constant variance. IQ tests are normalized to a standard distribution, so this assumption is valid for IQ. An analysis by Equilibrium Environmental Inc. (2008a) demonstrates that the Canadian SBP data follow an approximately normal distribution. The variance in SBP increases with increasing SBP (Canadian Heart Health Database, 1992), but the change in

variance over the change in SBP that is used in this analysis (1.1 to 1.4 mmHg) is sufficiently small that it does not have a significant influence on the results.

The concept of relating changes in the population distribution of IQ to changes in the prevalence of children with MMR (IQ< 70) or changes in the prevalence of "gifted" children (IQ> 130) has been well discussed elsewhere (Weiss, 1988; Weiss, 2000; Fewtrell *et al.*, 2004; Gilbert and Weiss, 2006). A shift in a population mean (from 100) of -1 IQ point is associated with an increase in the prevalence (added risk) of children with IQ < 70 of 17% (from 2,275 to 2,660 in 100,000) and a decrease in the prevalence of children with IQ >130 of 15% (from 2,275 to 1,938 in 100,000).

Normal distribution theory was used to model the following two dichotomous outcomes associated with a 1% increase in population mean SBP: (1) the extra risk of hypertension (Pre, Stage I and Stage II hypertension); and (2) the added risk of coronary heart disease (CHD) mortality. The methods and results of this modeling are presented in detail in Appendix A. The results are presented here to demonstrate the significance of a 1% change in population mean SBP as it relates to these outcomes.

The results of the normal distribution theory modeling and associated population rates presented in Appendix A are that:

- 1% decrement in population mean IQ is associated with an added risk of MMR of approximately 1 in 250 (385 in 100,000).
- The total sex and age-adjusted added risk of hypertension (Pre, Stage I and Stage II hypertension) among 35 to 74 year olds associated with a 1% increase in population mean SBP is approximately 1 in 20 (5,421 per 100,000).
- The sex and age-adjusted cumulative (35 to 74 years) incremental risk of CHD mortality associated with a 1% increase in population mean SBP is approximately 1 in

2,000 (50 per 100,000). Males are more sensitive than females and constitute about 80% of the modeled CHD deaths.

Fewtrell *et al.* (2004) has also estimated the risk of cardiovascular morbidity associated with lead related changes in population SBP; however, the modeling presented in Appendix A of this report is more sophisticated, is based on Canadian SBP and CHD mortality data, and extends the analysis to include lead-related CHD mortality. Rose (1981) has also analyzed the significance of small changes in population mean BP, and has estimated that reducing the population mean BP by 2 to 3 mmHg would be as effective in reducing the prevalence of hypertension as providing drug therapy for all clinically defined hypertensive individuals. It must be noted that the efficacy of drug therapy in managing hypertension has likely improved in the time since Rose (1981) conducted his analysis. Cook et al. (Cook *et al.*, 1995) estimated that a reduction in population mean DBP of 2 mmHg would result in a 17% decrease in the prevalence of hypertension, a 6% reduction in the risk of coronary heart disease, and a 15% reduction in the risk of stroke and transient ischemic attacks.

In summary, from a population health perspective, a 1% decrease in population average IQ and a 1% increase in population average SBP are associated with significant increases in population health risks.

6.3.3 Models of the Blood Lead Concentration Response-Relationships

As described above, no lead effect at blood lead concentrations of 0 μ g/dL was assumed and calculated the BMC associated with an absolute increase in SBP of 1.33 mmHg and an absolute decrement in IQ of 1 point. This required extrapolating the blood lead concentration response relationship from below the minimum blood lead concentrations included in the critical studies to a blood lead concentration of 0 μ g/dL. It was assumed that there was no population threshold for the critical effects because the weight of evidence fails to identify a population threshold for IQ and SBP.

In the context of this discussion, *low-dose linear* means that, at low doses, the response increases proportionally with an increase in dose, not that the dose-response function is linear over the entire range of exposures. In addition, the term *dose-response* defines dose broadly, and includes internal dose as measured by blood lead concentrations.

Study data was not fit with *de novo* blood lead concentration-response models for the IQ and SBP endpoints. Instead, the blood lead concentration-response models that were fit by the original study authors and published in the peer-reviewed literature were relied upon for these endpoints. For the children's blood lead-IQ relationship, a continuous blood lead concentration-response model was constructed from two models published in the critical study. One of the published models was a log-linear model based on all of the study data. The second published model was a linear model based only on the lower range of the study data. However, the log-linear model is not compatible with blood lead concentration-response estimates over relatively low ranges of blood lead concentrations (the slope of the log-linear model tends toward infinity as the blood lead concentration tends toward zero). Therefore, the two models were joined so that the maximum slope of the log-linear model would be limited to the slope of the lower exposure linear model.

Justification for Low-Dose Linear Dose-Response

US NRC (2008) Committee on Improving Risk Analysis Approaches used by the US EPA recommended that a low-dose linear model be assumed, unless there was sufficient data to reject low-dose linearity. The US NRC (2008) did not define what constitutes sufficient data, but in the case of blood lead and IQ, and blood lead and SBP, there is very little evidence of a population threshold.

The US NRC (2008) recommends two systematic assessments to support the determination of the conceptual shape of the low-dose shape of the population dose-response relationship: (1) a mode of action assessment; and (2) a background and vulnerability assessment. Both of these methods were given consideration, but it was concluded that they provided no definitive determination on the presence or absence of a population threshold for the critical effects of lead. The approach of the US NRC (2008) is built upon the premise that interindividual variability in susceptibility and background additivity will produce a low-dose linear population dose-response. The mode of action assessment and background and vulnerability assessment are provided as a systematic means of reviewing the available evidence of interindividual variability and background additivity. The noted objections to this approach are twofold: (1) the US NRC (2008) does not provide guidance for conducting these assessments or offer decision-making criteria; and (2) the required analyses would be onerous, complex, uncertain and ultimately speculative, at least in the case of lead and the critical endpoints examined. Evidence of background additivity and inter-individual susceptibility does not establish that there will not be a minimum level of population lead exposure that can be tolerated without producing a measurable population effect on the critical endpoints (IQ and SBP).

There are several modes of action by which lead can cause adverse effects on neurodevelopment and blood pressure. Many of these modes of action are also induced by other chemical exposures or endogenous processes. A good example is oxidative stress: Lead exposure results in increased production of reactive oxygen species and oxidative stress. Three oxidative stress mediated modes of action (inhibition of NO, inhibition of sGC, and activation of NF-κB) for the hypertensive effects of lead are well supported by *in vitro* and *in vivo* evidence at environmentally relevant lead exposures (Vaziri and Khan, 2007). The events (or background processes) that these oxidative stress mediated perturbations contribute to include vasoconstriction, salt retention, activation of the sympathetic nervous system, stimulation of the renin-angiotensin system, and platelet adhesion. These, in turn,

contribute to endothelial dysfunction, hypertension, inflammation, arteriosclerosis, and thrombosis. Oxidative stress, of course, is on the etiological pathway for other diseases or disease processes, such as neurodevelopmental effects, neurodegeneration, renal dysfunction, immune dysfunction, and cancer. By this cursory analysis, the oxidative stress mediated adverse effects of lead meet the US NRC's (2008) criteria to be classified as a population low-dose linear toxicant in two ways: (1) lead contributes to an independent "background" disease process; and (2) the background process results in a relatively high incidence of disease (> 1%). However, this does not demonstrate that there is not some incremental level of lead exposure that the population can tolerate without producing a significant increase in response (e.g., increased blood pressure).

While such a complex mechanistic and theoretical approach may or may not support the argument that the adverse effects of lead on IQ and SBP are expected to be low-dose linear on a population basis, adverse effects on IQ and SBP have been observed at the lowest exposures studied, in both humans and experimental animals. In the case of IQ, the preponderance of evidence suggests that the exposure-response relationship becomes stronger, rather than weaker, over the lower ranges of exposures studied in environmental epidemiology. This provides a relatively high degree of certainty that the dose-response relationships continue below the ranges of exposure yet studied. While it remains uncertain for how low the dose-response relationship will carry on, in the absence of evidence to the contrary, the most neutral assumption is that the blood lead concentration-response relationships continue in a linear fashion at a similar magnitude (slope) as that indicated by the lower range of the empirical evidence. Therefore a linear extrapolation of the blood lead concentration relationships for IQ and SBP were judged supportable both in theory and based on the available empirical data. The methods by which the slopes were determined are described below.

6.3.4 Blood Lead and IQ

The only parameter from the blood lead concentration-response models that was required to calculate the benchmark blood lead concentrations was the slope of the model. In all cases, a dose-response model was not fit, *de nova*, to the data of the critical study; rather, the published adjusted regression coefficients from the published studies were used. The slopes of these adjusted regression coefficients were assumed to extend to the origin.

In the case of the critical study for IQ, the regression model of the full data set is a log-linear model, and the slope of this model cannot be extrapolated over lower blood lead concentrations. This is because thebecuase the model predicts an ever-increasing slope to the blood lead concentration-IQ relationship and the function is undefined at a blood lead concentration of 0 µg/dL. Four options were considered in determining the most defensible method of defining the slope of the blood lead concentration. The objective in evaluating these options was to determine the method that best reflected the blood lead concentration-response from the pooled data set and minimized or eliminated dependence on subjective decisions to make the model compatible with low-dose extrapolation. The four options considered were:

Secant: This method derives a linear approximation of the slope of the log-linear model over a specified range of blood lead concentrations by constructing a secant (a line joining two points on a curve). An example of the secant option is provided in Lanphear *et al.* (2005), where, based on their adjusted log-linear model, the authors provide an estimate of the decrement in mean population IQ over the interval of 2.4 to 10 μ g/dL of 3.9 IQ points. The slope of this secant is -0.5 IQ points per μ g/dL increase in blood lead. Lanphear *et al.*

(2005) chose to estimate the change in IQ over this interval because $2.4^{13} \mu g/dL$ was the 5th percentile of their data and 10 $\mu g/dL$ is the US CDC's current lowest blood lead intervention level. The secant described by Lanphear *et al.* (2005) over the range of concurrent blood lead of 2.4 to 10 $\mu g/dL$ is illustrated in Figure 29 as a dashed line connecting the points x_2 and x_3 . A secant provides a measure of the average slope of the log-linear response over a specified range of blood lead concentrations, but overestimates the log-linear response over the upper half of the blood lead concentration range and underestimates the log-linear response over the lower half of the blood lead concentration range. The slope of a secant derived from any two points on the log-linear curve could be used to provide a linear low-dose extrapolation of the blood lead concentration-IQ relationship. The slope of a secant is dependent on the choice of the two points on the curve from which the secant is derived.

Tangent: This method derives a linear estimate of the slope by calculating a tangent to the log-linear model at a specified blood lead concentration. For example, the tangent to the slope of the Lanphear *et al.* (2005) adjusted log-linear model at 2.4 μ g/dL (the 5th percentile of concurrent blood lead in their study data) has a slope of approximately -1.1 IQ points per μ g/dL increase in blood lead. The tangent to the Lanphear *et al.* (2005) adjusted log-linear model at 2.4 μ g/dL is illustrated in Figure 29 as a dashed line below the point x₂. A tangent is equal to the slope of the log-linear model at the point of the tangent, but is an over-estimate of the log-linear response at higher blood lead concentrations. The slope of a tangent derived from any point on the log-linear curve could be used to provide a linear low-dose extrapolation of the blood lead concentration-IQ relationship. The slope of a tangent is dependent on the choice of the point on the curve from which the tangent is derived.

¹³ As reported earlier, the 5th percentile of concurrent blood Pb was corrected to 2.5 μ g/dL. The difference between slopes of the secants between 2.4 and 10 μ g/dL and 2.5 and 10 μ g/dL is not significant in the context of this discussion.

The slope of a curved function at a point (a tangent) can be found by differentiation. The first derivative (equivalent to the slope of a tangent to the curve) of the function $-2.7\ln(x)$ can be determined for any point on the curve (x) by Equation 4.

Equation 4

$$\frac{dy}{dx} - 2.7 \ln(x) = -2.7 \times \frac{dy}{dx} \ln(x)$$
$$= -2.7 \times \frac{1}{x}$$
$$= \frac{-2.7}{x}$$

Segmented Linear: The third option considered was to simply adopt the linear model published by Lanphear et al. (2005) for subjects with peak blood lead less than 7.5 µg/dL. The slope (and 95th percent confidence intervals) for the linear model for subjects with a peak blood lead of less than 7.5 µg/dL was -2.94 (-0.17 to -5.16) IQ points per µg/dL increase in blood lead. The segmented linear model is illustrated in Figure 29. In their review of the US EPA's draft risk assessment for the National Ambient Air Quality Standard (NAAQS) for lead, the US EPA's Clean Air Scientific Advisory Committee (CASAC) recommended use of the linear concentration-response function derived from the subset of children in the Lanphear et al. (2005) pooled analysis with measured peak blood lead concentrations less than 7.5 µg/dL for low-dose extrapolation (Henderson, 2007). Alternatively, the California Environmental Protection Agency chose to use the linear concentration-response function derived the subset of children in the Lanphear et al. (2005) pooled analysis with measured peak blood lead concentrations less than 10 µg/dL in support of calculating their soil screening level (California Environmental Protection Agency, 2007). As a point of comparison, subsets of children from the Rochester (n = 101) and Boston (n = 48) cohorts whose peak measured blood lead concentrations never exceeded 10 µg/dL had adjusted linear regression coefficients of -1.8 and -1.6 IQ points per µg/dL (concurrent for Rochester and two-year-old

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blood lead for Boston) (Bellinger and Needleman, 2003b; Canfield *et al.*, 2003a). The slope of a segmented linear model is dependent on the choice cut-point to stratify the data.

Log-linear with Low Exposure Linearization: This option is a two-piece model that is linear below a specified cut-point and log-linear above. The slope of the low exposure linear model is equivalent to the tangent of the log-linear model at the cut-point. The US EPA developed a two-piece model of this form in their 2007 risk assessment for the National Ambient Air Quality Standards (NAAQS) for lead and called the model "log-linear with low exposure linearization" (US EPA, 2007c). The same term has been used for the version of this model presented herein. The slopes of this two-piece model are illustrated in Figure 29, with a linear slope at and below the point x_1 and a log-linear slope above x_1 . The log-linear with low exposure linearization model will underestimate the log-linear component of the log-linear with low exposure linearization model is dependent on the choice of cut-point where the low exposure linear model is joined to the log-linear model.

Two data-derived methods of determining the cut-point for the log-linear with low exposure linearization model were considered: The first method, which was also used by the US EPA (2007) in their NAAQS risk assessment, is to set the cut-point equal to the minimum of the data used to generate the log-linear model. However Lanphear *et al.* (2005) did not publish the minimum range of their concurrent blood lead data. While the US EPA (2007b) chose a value of 1 μ g/dL for this cut-point, Hornung (pers com. 2009) reported that the minimum concurrent blood lead in their data was 0.5 μ g/dL. The slopes of tangents at cut-points of 1 and 0.5 μ g/dL are -2.7 and -5.4 IQ points per μ g/dL increase in blood lead, respectively.

While the rationale of using the minimum of the study data to determine the cut-point for the two-piece linear model was understood, and to a certain extent supported,, there was some concern that the slope of the linear model below $0.5 \ \mu g/dL$ exceeded the measured linear

slope for subjects with peak blood lead concentrations of less than 7.5 μ g/dL. Therefore it was decided to limit the slope of the low-dose linearization to the measured linear slope for subjects with peak blood lead concentrations less than 7.5 μ g/dL. The slope of this model was -2.94 IQ points per μ g/dL increase in blood lead. The point at which the slope of a tangent to the log-linear model is equivalent to -2.94 IQ points per μ g/dL is 0.92 μ g/dL. Therefore, 0.92 μ g/dL was selected as the cut-point for the log-linear (with low dose linearization) model; this value was then rounded to 0.9 μ g/dL, because concurrent blood lead was only reported to a tenth of a μ g/dL in the original publication. The slope of this two-piece model is illustrated in Figure 29, with a linear slope of -2.94 IQ points per μ g/dL increase in blood lead at and below 0.9 μ g/dL (point x₁) and a log-linear slope of -2.7 IQ points per natural log μ g/dL increase in blood lead above 0.9 μ g/dL.

Options 1,2 and 4 are illustrated in Figure 29.



Figure 29. Slope of the Lanphear et al. (2005) log-linear model of concurrent blood lead and IQ, and options for deriving a linear estimate of the log-linear slope. The solid curved line is the slope of the adjusted log-linear model from Lanphear et al. (2005) with an intercept of 100 for purposes of illustration. Various options for deriving a linear estimate of the log-linear model are illustrated with dashed lines. The point x_2 is at a concurrent blood lead of 2.4 μ g/dL, the 5th percentile of the concurrent blood lead data in the pooled analysis. The point x_3 is at 10 μ g/dL. The

point x_1 is at 0.9 μ g/dL, the point at which the tangent to the log-linear slope is equivalent to the adjusted linear slope of subjects with peak blood lead less than 7.5 μ g/dL. The dashed line ending at x_1 is the low-dose linearization option with a cut-point of 0.9 μ g/dL. The dashed line connecting x_2 and x_3 is the secant option over the range of 2.4 to 10 μ g/dL. The dashed line ending at x_2 is the tangent option at a blood lead of 2.5 μ g/dL.

Of the options considered, it is recommended that the quantitative estimate of the blood lead-IQ blood lead concentration-response relationship be based on the log-linear model with lowexposure linearization (option 4).

Recommending a functional-form of the blood lead-IQ relationship that is compatible with the low dose extrapolation required for the benchmark dose method involves weighing the relative merits and deficiencies of the available options.

An alternative to the secant and tangent options is to simply use the published low-exposure linear model of the truncated data. However, the low-exposure linear model was based on less than 10% of subjects included in the pooled analysis, and this subset of subjects was comprised primarily of those from the Rochester cohort. While Lanphear *et al.* (2005) provided results of regression diagnostics and sensitivity analysis for the log-linear model based on the full data set, no similar supporting evidence was published for the low-exposure linear model. This raises questions about the degree to which the low-exposure linear model might have been overly dependent on the data from the Rochester cohort and how representative these data are for the full spectrum of ethnic, economic, and social diversity of the Canadian population. The use of the low-exposure linear model has the added shortcoming that it will tend to over-estimate the blood lead concentration-response relationship over relatively higher blood lead concentrations. Finally, the slope of the low-exposure linear model is also dependent on the choice of cut-point. This is illustrated by the difference in slopes between the linear model for subjects with peak blood lead less than 7.5 μ g/dL ($\beta_1 = -2.94$) and the linear model for subjects with peak blood lead less than

10 μ g/dL (β_1 = -0.80) (Lanphear *et al.*, 2005). For these reasons, there is lower confidence in the low-exposure linear model than the models based on the entire pooled data set.

The secant, tangent and low-dose linearization options have the advantage of being based on the fully developed log-linear model for the entire pooled data set. However, the slopes of all are dependent on the selection of a point or points on the log-linear curve. The least arbitrary of the available options that are based on the log-linear curve from the full data set is the low-dose linearization option where the magnitude of the slope of the linear component is limited to the magnitude of the segmented linear slope from the critical study. The loglinear model with low-exposure linearization (option 4) is also the only one of the linear options that does not tend to over-predict the log-linear relationship at higher exposures.

The log-linear model with low-exposure linearization (option 4) with a cut-point set at $0.9 \mu g/dL$ represents a compromise between the use of the published segmented linear model, based with its relatively limited data and sensitivity analysis, and the use of a linear approximation of the full log-linear model that is dependent on the choice of points on the curve. Of the options requiring selection of points on the curve, the log-linear model with low-exposure linearization (option 4), requires the least arbitrary selection of a point. As discussed above, a point on the curve where the tangent was equal to the slope of the adjusted linear model for subjects with peak blood lead less than 7.5 $\mu g/dL$ was selected. This represents a justifiable and empirically based limit to the ever-increasing slope of the log-linear model as blood lead concentrations decline. The log-linear model with low-exposure linearization also presents a neutral approximation of the blood lead concentration-response relationship when extrapolated below the range of study data (i.e., it is not supralinear nor sublinear over the range of extrapolation).

There is some uncertainty with respect to how low the log-linear model from Lanphear *et al.* (2005) represents an unbiased and valid representation of the blood lead concentration-IQ

relationship. This is a critical issue, as the potential bias of the log-linear model rapidly increases at blood lead concentrations less than the lowest point at which the log-linear model provides a valid estimate of the true underlying relationship. It was assumed that the log-linear model provides a valid and unbiased estimate of the underlying relationship down to a minimum blood lead concentration of 0.9 (0.7 to 2.3) μ g/dL. These values are within the range of blood lead concentrations included in the study data, but they are very close to the minimum of the data (0.5 μ g/dL). Therefore, a log-linear model with low-dose extrapolation with the cut-point set at 2.5 μ g/dL, 5th percentile of the concurrent blood lead data of the Lanphear *et al.* (2005), was also constructed. This secondary model was constructed to illustrate the sensitivity of the benchmark modeling results to the assumption that the log-linear model represents an unbiased and valid representation of the blood lead concentration of the minimum range of data in the study.

Blood Lead and IQ Summary

As the log-linear model derived from the full data set of the critical study of Lanphear *et al.* (2005) is not compatible with the requirements of BMD modeling, several options for providing an estimate of the slope of the blood lead-IQ relationship for low-dose extrapolation were examined. It was determined that the option least dependent on arbitrary choices is a two-piece model that is linear in the low dose region and is based on the log-linear model of the full data set elsewhere. The cut-point at which the linear slope is joined to the log-linear slope is defined as the point where the tangent to the log-linear model is equal to the slope of the adjusted linear regression coefficient from the published linear regression of subjects in the critical study who had maximum blood lead concentrations of less than 7.5 μ g/dL. This approach is consistent with two of this report's objectives: (1) using only published regression coefficients as the slopes in the BMD models; and (2) using a method that best reflects the blood lead concentration-response from the pooled data set and minimizes or eliminates dependence on any subjective decisions to make the model compatible with low-dose

extrapolation. Therefore, the slope of the blood lead concentration-IQ relationship used in the BMD modeling is linear with a slope of -2.94 (-0.71 to -5.16) IQ points per μ g/dL below a cutpoint of 0.9 (2.3 to 0.3) μ g/dL and is log-linear above the cut-point with a slope of -2.70 (-1.66 to -3.74) IQ points per natural log μ g/dL. These slopes are illustrated in Figure 30 and summarized in Table 21 below. BMD modeling was also conducted with low dose-linearization of the log-linear slope at a cut-point of 2.5 μ g/dL to illustrate the sensitivity of the results to the assumption that the log-linear slope represents an unbiased estimate of the underlying relationship down to the lower cut-points.

In Section 5 it was recommended that the regression slope of the log-linear model from Lanphear *et al.* (2005) be used as an estimate of the slope of the blood lead concentration-IQ relationship for blood lead concentrations greater than 1 μ g/dL. It is important to note that the upper confidence interval of the slope of the log-linear low-dose linearization slope used in the BMD modeling is linear over the range of 1 to 2.3 μ g/dL. This means that the upper confidence limit of the slope used for BMD modeling is shallower than the log-linear slope recommended in Section 6. The maximum difference in estimated IQ as a function of blood lead over this range is 0.4 IQ points. The magnitude of this difference is small relative to the overall uncertainty of the estimated slope, but risk assessors should be aware of this potential discrepancy and the reason for it.



Figure 30. Slopes of the relationship between children's blood lead and population mean IQ used for BMD modeling. The middle line is the best estimate of the slope, and it is bounded by upper and lower estimates of the slope. The dashed lines indicate the regions where the slopes have been extrapolated to blood lead concentrations beyond the range of study data. The progressive shading indicates the region where the slopes have been extrapolated below the range of study data and indicates increasing uncertainty in the estimates of the slopes. The constant dashed line at a change in IQ of -1 represents the benchmark response and the intercepts between this constant and the slopes of the blood lead-SBP relationship define the benchmark blood lead concentrations.

Estimate	Slope ¹ of Low Dose	Cut-Point ²	Slope ¹ of Log-Linear
	Linear		
Upper estimate	-0.71	2.3	-1.66
Best estimate	-2.94	0.9	-2.70
Lower estimate	-5.16	0.7	-3.74

Table 21. Maximum likelihood and upper and lower estimates of the slope of the relationship between population mean IQ and concurrent blood lead in children.

1. The slopes for the low dose linear components are the reported maximum likelihood and 95^{th} percent confidence intervals of the adjusted linear model for subjects in the Lanphear *et al.* (2005) pooled analysis with a maximum concurrent blood lead concentration of < 7.5 µg/dL. The slopes for the log-linear components are the reported maximum likelihood and 95^{th} percent confidence intervals of the adjusted log-linear model for all subjects in the Lanphear *et al.* (2005) pooled analysis.

2. The cut-points were calculated by determining the blood lead concentration at which the tangent to the slope of the log-linear relationship was equal to the adjusted linear regression coefficient for subjects from the pooled analysis with a maximum blood lead concentration of $< 7.5 \mu g/dL$.

6.3.5 = Blood Lead and Systolic Blood Pressure (SBP)

The regression models for the critical studies of blood concentrations and SBP are both linear models. Therefore, the adjusted regression coefficients from the published linear models can be used without modification, as the slope parameter in the benchmark dose model for calculating risk-specific blood lead concentrations. The slopes of these models are simply extrapolated to the origin for the BMD modeling and calculations.

The parameters for the upper and lower estimates of the slope of the relationship between population mean SBP and blood lead in adults are presented in Table 22 below. These slopes are illustrated in Figure 31.

Table 22. Summary of recommended slopes of the relationship between blood lead in adults and population mean systolic blood pressure.

Endpoint	Critical Study	Slope
Lower estimate, Caucasian males	Glenn <i>et al.</i> (2003)	0.05 mmHg per µg/dL
Best estimate, Caucasian males	Glenn <i>et al.</i> (2003)	0.25 mmHg per µg/dL
Best estimate, susceptible sub- populations	Vupputuri <i>et al.</i> (2003)	0.47 mmHg per µg/dL
Upper estimate, susceptible sub- populations	Vupputuri <i>et al.</i> (2003)	0.80 mmHg per μg/dL



Figure 31. Slopes of the relationship between adult blood lead and population mean systolic blood pressure used for BMD modeling. The dark solid lines are the recommended best estimates of the slopes for Caucasian males and susceptible sub-populations. The light solid lines are the lower and upper estimates of the slopes. The dashed lines indicate the regions where the slopes have been extrapolated to blood lead concentrations beyond the range of study data. The progressive shading indicates increasing uncertainty in the estimates of the slopes. The slopes have not been tested for non-linearity; $4 \mu g/dL$ is the approximate mid-point of the data for the critical studies. The constant dashed line at a SBP of 1.3 mmHg indicates the benchmark response and the intercepts between this constant and the slopes of the blood lead-SBP relationship define the benchmark blood lead concentrations.

6.3.6 Benchmark Blood Lead Concentrations

The general form of the equation used to calculate benchmark blood lead concentrations is described in Equation 5. The parameters required for this model are: (1) the benchmark response (BMR); and (2) the slope of the blood lead concentration-response relationship. The methods used to derive these parameters and their values are provided in the preceding sections.

Equation 5

$$BMC = \frac{BMR}{\beta_1}$$

where BMC = Benchmark blood Pb Concentration (µg/dL) BMR = Benchmark response (1.3 mmHg for SBP and 1 IQ point for IQ) $\beta_1 =$ slope of the blood Pb concentration - response relationship

In the case of IQ, a slightly different approach is required to accommodate the joined linear and log-linear slopes. A BMC is calculated via Equation 6 when the BMR is greater than or equal to the slope of the log-linear relationship (e.g., when the BMR is \geq -1.66, -2.70, or - 3.74). For example; to calculate the benchmark blood lead concentration for a benchmark response of -1 IQ point, Equation 6 was used for all estimates because -1 \geq -1.66, -2.70, and -3.74. Note that the slope (β_1) in Equation 6 is the adjusted regression coefficient for the linear model for subjects with maximum blood lead concentrations of less than 7.5 µg/dL from the critical study of Lanphear *et al.* (2005).

Equation 6

$$BMC = \frac{BMR}{\beta_1}; BMR \ge \beta_1 \times C$$

where BMC = Benchmark blood Pb concentration (µg/dL) BMR = Benchmark response (-1 IQ point) β_1 = adjusted linear regression coefficient : best estimate = -2.94; lower estimate = -5.16; upper estimate = -0.71 (IQ points per µg/dL) C = Cut - point : best estimate = 0.92; lower estimate = 0.72; upper estimate = 2.34 (µg/dL)

A BMC is calculated via Equation 7 where the BMR is less than the slope of the log-linear relationship (e.g., when the BMR is < -1.66, -2.70, or -3.74). For example; to calculate the benchmark blood lead concentration for a benchmark response of -4 IQ points, use Equation 7 for all estimates because -4 < -1.66, -2.70, and -3.74. Note that the slope (β_1) in Equation 7 is the adjusted regression coefficient for the log-linear model for the entire data set of the critical study of Lanphear *et al.* (2005).

Equation 7

 $BMC = e^{\left(\frac{BMR - \beta_1 + [\beta_1 \times \ln(C)]}{\beta_1}\right)}; BMR < \beta_1$ where $\beta_1 = \text{adjusted log - linear regression coefficient : best estimate = -2.70;}$ lower estimate = -3.74; upper estimate = -1.66 (IQ points per natural log µg/dL)

In the case of SBP, BMCs were calculated via Equation 8.

Equation 8

$$BMC = \frac{BMF}{\beta_1}$$

where BMC = Benchmark blood Pb Concentration (µg/dL) BMR = Benchmark response (1.3 mmHg) $\beta_1 =$ adjusted linear regression coefficient : best estimate Caucasian males = 0.25; lower estimate Caucasian males = 0.05; best estimate susceptible sub - populations = 0.47; upper estimate susceptible sub - populations : 0.80 (mmHg per µg/dL)

6.3.7 Risk-Specific Blood Lead Concentrations

The benchmark blood lead concentrations define a single blood lead concentration associated with a specified level of response. When exposure, whether expressed as lead intake or a blood lead concentration, is measured or estimated for a risk assessment the exposure will be expressed as a distribution or as a point estimate on a distribution. To provide a useful and transparent benchmark of toxicity for risk characterization, the TRV should also be expressed as a distribution or explicitly identified as a point estimate on a distribution. Therefore a population distribution of blood lead concentrations was assumed and the risk-specific blood lead concentrations (i.e., the TRVs) were defined as the *geometric mean* of a population blood lead concentration (BMC). This allows the population risk associated with the risk-specific blood lead concentration to be described probabilistically as the geometric blood lead concentration in a population where the health risk for 95% of the population is less than the benchmark response (BMR). This concept is illustrated in Figure 32.

The ratio of the 95th percentile to the geometric mean blood lead concentration was calculated from data reported by recent nationally representative biomonitoring studies. The

BMCs were divided by the mean of this ratio to produce the risk-specific blood lead concentrations.

Figure 32. Conceptual illustration of a benchmark blood lead concentration (BMC) and an associated risk-specific blood lead concentration. The BMC is calculated from a benchmark response and the slope of the relationship between blood lead and the response. The BMC is divided by the ratio of the 95th percentile to the geometric mean blood lead concentrations reported in nationally representative biomonitoring studies. The quotient is the risk-specific blood lead concentration. A population with a geometric mean blood lead concentration equal to the risk-specific blood lead concentration will have no more than a 5% probability of health risks equivalent to the benchmark response.



6.4 RESULTS

The following sections presents the results from calculating the benchmark blood lead concentrations and the risk-specific blood lead concentrations.

6.4.1 Benchmark Blood Lead Concentrations

Blood Lead-IQ

The benchmark blood lead concentrations (BMCs) associated with various benchmark responses (BMRs) in mean population IQ are presented in Table 23. The equations and parameters for calculating the BMCs are presented in Section 6.3 above. The BMCs associated with a BMR of -1 IQ point in population mean IQ are shown in bold. The best estimate BMC associated with a 1 point decrement in mean IQ is 0.3 μ g/dL, with a lower estimate BMC of 0.2 μ g/dL and an upper estimate BMC of 1.4 μ g/dL. The lower and upper estimates of the BMC reflect some, but not all of the variability and uncertainty in the slope of the blood lead concentration-IQ relationship. BMCs for other BMRs are shown for illustration.

Table 23. Benchmark blood lead Concentrations (BMCs) (µg/dL) corresponding to various benchmark responses (BMRs) of change in mean population IQ. BMCs are provided for the best, lower, and upper estimates of the blood lead concentration-response relationships that were derived from the international pooled analysis of Lanphear et al. (2005). The BMC values in the shaded cells were calculated using Equation 7; the balance of the values were calculated using Equation 6.

	Lower	Best	Upper
	Estimate	Estimate	Estimate
	BMC	BMC	BMC
BMR (IQ points)	(µg/dL)	(µg/dL)	(µg/dL)
-0.5	0.1	0.2	0.7
-1.0	0.2	0.3	1.4
-1.5	0.3	0.5	2.1
-2.0	0.4	0.7	2.9
-3.0	0.6	1.0	5.2
-4.0	0.8	1.5	9.6
-5.0	1.0	2.2	17.5

As discussed above, there is some uncertainty about whether the lower extent of blood lead concentrations over which the adjusted log-linear model of the relationship between concurrent blood lead and IQ from the critical study of Lanphear *et al.* (2005) represents a valid and unbiased estimate of the true underlying relationship. The potential bias in the estimate of the log-linear model increases exponentially below the point where the model is no longer a valid reflection of the true underlying relationship. For the primary analysis above,
the log-linear model of Lanphear *et al.* (2005) was assumed valid and unbiased down to blood lead concentrations as low as 0.7 μ g/dL. Although this is within the range of the study data, it is very close to the minimum concurrent blood lead concentration data (0.5 μ g/dL). Therefore, a secondary set of BMC calculations were conducted as a sensitivity analysis. For the secondary BMC calculations, the log-linear model was assumed valid down to a blood lead concentration of 2.5 μ g/dL, which is the 5th percentile of the concurrent blood lead data in the pooled analysis. The secondary BMC calculations were made based on a low-dose linear extrapolation where the slope of the low-dose linear component was equal to the tangent of the log-linear model at a blood lead concentration of 2.5 μ g/dL. The results of this sensitivity analysis are presented in Table 24. The assumption that the log-linear model is unbiased down to a blood lead concentration of 0.7 μ g/dL makes about a three-fold difference in BMCs relative to an assumption that the log-linear model provide an unbiased estimate down to a blood lead concentration of 2.5 μ g/dL. The uncertainty of this assumption is contained within the quantitative upper and lower estimates of the BMC that were developed in the primary BMD model. Table 24. Benchmark blood lead Concentrations (BMCs) (μ g/dL) corresponding to a benchmark response (BMRs) of a 1 point decrement in mean population IQ. BMCs are provided for 1) a log-linear model that extends down to cutpoints where the tangent of the log-linear model is equal to the adjusted regression coefficient of the linear regression model of subjects in the critical study who had maximum blood lead concentrations of less than 7.5 μ g/dL (primary model); and 2) a log-linear model that extends down to cut-points at 2.5 μ g/dL (secondary model).

			Lower	Best	Upper
			Estimate	Estimate	Estimate
			BMC	BMC	BMC
Model	Parameter	Units	(µg/dL)	(µg/dL)	(µg/dL)
Primary model	Cut-points	µg/dL	0.7	0.9	2.3
	Slope of low-dose	IQ per μg/dL	5 16	2.04	0.71
	Ineanzation		-5.10	-2.94	-0.71
	BMC	µg/dL	0.2	0.3	1.4
Secondary model	Cut-points	µg/dL	2.5	2.5	2.5
	Slope of low-dose linearization	lQ per µg/dL	-1.50	-1.08	-0.66
	BMC	µg/dL	0.7	0.9	1.5

Blood lead-SBP

The benchmark blood lead concentrations (BMCs) associated with various benchmark responses (BMRs) in population mean SBP are presented in Table 25. The equations and parameters for calculating the BMCs are presented above. The BMCs associated with a BMR of an increase in population mean SBP of 1.3 mmHg are shown in bold. The best estimate BMC associated with a 1.3 mmHg increase in population mean SBP is 2.7 μ g/dL for susceptible sub-populations and 5.0 μ g/dL for Caucasian males. These estimates are bounded by a lower estimate of 1.6 μ g/dL and an upper estimate of 25 μ g/dL. The lower and upper estimates of the BMC reflect some, but not all of the variability and uncertainty in the slope of the blood lead concentration-SBP relationship. BMCs for other BMRs are shown for illustration.

Table 25. Benchmark blood lead Concentrations (BMCs) (μ g/dL) corresponding to various benchmark responses (BMRs) of change in mean population systolic blood pressure. BMCs are provided for the following estimates of the blood lead concentration-response relationships: lower estimate for susceptible sub-populations, best estimate for Caucasian males, and an upper estimate for Caucasian males. The slopes were derived from the critical studies of Glenn et al. (2003) and Vupputuri et al. (2003).

	BMC	BMC		
	(µg/dL):	(µg/dL):	BMC	BMC
	Susceptible	Susceptible	(µg/dL):	(µg/dL):
	Sub-	Sub-	Caucasian	Caucasian
	populations	populations	Males	Males
	Upper	Best	Best	Lower
	Estimate of	Estimate of	Estimate	Estimate of
BMR (mmHg)	Slope	Slope	of Slope	Slope
0.5	0.6	1.1	2.0	10.0
1.0	1.3	2.1	4.0	20.0
1.3	1.6	2.7	5.0	25.0
1.5	1.9	3.2	6.0	30.0
2.0	2.5	4.3	8.0	40.0
3.0	3.8	6.4	12.0	60.0
4.0	5.0	8.5	16.0	80.0
5.0	6.3	10.6	20.0	100.0

6.5 RISK-SPECIFIC BLOOD LEAD CONCENTRATIONS

Risk-specific blood lead concentrations were derived by estimating the geometric mean of the population blood lead distribution that has a 95th percentile equivalent to the benchmark blood lead concentrations (BMCs).

An estimate of the ratio of the 95th percentile to the geometric mean blood lead concentration was derived from examining the ratio of these values for nationally representative biomonitoring data. These data and their respective ratios of the 95th percentile to the geometric mean blood lead concentration are presented in Table 26. The average of the ratios was 2.88. This value was rounded to 3 and the BMCs were then divided by 3 to

calculate the risk-specific blood lead concentrations. Risk-specific blood lead concentrations were calculated using Equation 9.

Equation 9

$$RSC = \frac{BMC}{95^{th}:GM}$$

where

RSC = the Risk - Specific Concentration, expressed as a population geometric mean

blood Pb concentration ($\mu g/dL$)

BMC = the Benchmark blood Pb Concentration (μ g/dL)

 95^{th} : *GM* = the ratio of the 95th percentile to geometric mean of the population blood Pb distribution (3)

Table 26. Geometric mean, 95th percentile, and ratio of 95th percentile to geometric mean to from nationally representative population biomonitoring studies of blood lead concentrations.

		Geomean			Source of log-normal
		or	95 th	Ratio of 95 th	distribution parameter
Life-stage	Sex	Median	Percentile	Percentile:geomean	estimates
Children 6-19	ð & ♀	0.88	2.05	2.33	CHMS GM and 95 percentile (Wong & Lye, 2008)
Children 1-5	ð & ♀	1.70	5.80	3.41	NHANES 2001-02 GM and 95 percentile (CDC, 2005)
Adults	ð & ♀	1.5	4.11	2.74	CHMS GM and 95 percentile (Wong & Lye, 2008)
All ages	ð & ♀	1.37	3.87	2.82	CHMS GM and 95 percentile (Wong & Lye, 2008)
All ages	5	1.78	5.30	2.98	NHANES 2001-02 GM and 95 percentile (CDC, 2005)
All ages	Ŷ	1.19	3.6	3.03	NHANES 2001-02 GM and 95 percentile (CDC, 2005)
Average				2.88	

The risk-specific blood lead concentrations are presented in Table 27. The best estimate of the children's blood lead concentration where the mean lead-associated IQ decrement will be no more than 1 IQ point in 95% of the population is 0.1 μ g/dL with a lower and upper estimate of 0.1 to 0.5 μ g/dL, respectively. The best estimate of the adult blood lead

concentration where the mean lead-associated increase in SBP will be no more than 1.3 mmHg in 95% of the population is 1.7 μ g/dL for Caucasian males and 0.9 μ g/dL for susceptible sub-populations. The lower and upper estimates of the adult blood lead concentration where the mean lead-associated increase in SBP will be no more than 1.3 mmHg in 95% of the population are 0.5 and 6.3 μ g/dL, respectively.

	Units	Lower			
IQ		Estimate	Best Estimate	Upper Estimate	
BMR	IQ points	-1	-1	-1	
BMC	µg/dL	0.2	0.3	1.4	
Ratio 95th:GM	none	3	3	3	
Risk-specific blood lead concentration	µg/dL	0.1	0.1	0.5	
		Lower	Best estimate	Best estimate	Upper
SBP		estimate	susceptible	Caucasian males	estimate
BMR	mmHg	1.3	1.3	1.3	1.3
BMC	µg/dL	1.6	2.7	5	25
Ratio 95th:GM	none	3	3	3	4
Risk-specific blood lead concentration	µg/dL	0.5	0.9	1.7	6.3

Table 27. Risk-specific blood lead concentrations for the critical endpoints IQ and SBP

6.5.1 Potential *in Utero* and Translactational Lead exposures

The proportion of a women's body burden of lead that is remobilized during pregnancy and lactation and potentially transferred to the fetus and infant is uncertain (Equilibrium Environmental Inc., 2008b). However, several longitudinal birth cohort studies have reported that cord blood lead and maternal blood lead are well correlated. The blood lead concentration-response relationship between maternal blood lead and IQ decrements in offspring is also not sufficiently well established to allow for the development of a separate risk-specific blood lead concentration for women of childbearing age that would infer equal

protection to the offspring as the risk-specific blood lead concentration that has been developed based on the blood lead concentration-response relationship for children. However, there is insufficient evidence and a lack of logic to support the conclusion that the fetus would be less vulnerable than a child to the developmental neurotoxic effects of lead. Therefore, it is recommended that the risk-specific blood lead concentration for IQ endpoint also apply to prenatal exposures and, by extension, to women of childbearing age. Ergo, the risk-specific blood lead concentrations and IQ also applies to adult women of childbearing age (19 to 45 years old).

6.6 SUMMARY

This section of the report:

Provides a rationale for deriving a probabilistic risk-specific blood lead concentrations rather than the attempting to derive a "threshold" toxicological reference value (TRV) for lead. TRVs for non-carcinogens have traditionally been defined as a "threshold" below which there is no risk of adverse effects. There are, however, fundamental and operational issues with this traditional approach. Fundamentally, the threshold TRV approach does not allow exposure risks to be characterized quantitatively. Non-cancer risks have been traditionally characterized qualitatively as either "acceptable" or "unacceptable". Operationally, existing data does not provide strong evidence of a threshold for the critical effects of lead and there is rationale to suggest that these effects may not have a threshold when viewed from a population perspective. Therefore, the TRVs for lead derived in this report are defiend as blood lead concentrations associated with some *a priori* defined probability of effect. This approach was taken because currently available data do not identify, with confidence, a blood lead concentration that is without risk of adverse population effects.

Illustrates the use of a benchmark dose (BMD) approach to calculate benchmark blood lead concentrations (BMCs). The BMD approach uses the blood lead concentrationresponse slopes from the published critical studies to calculate the blood lead concentrations associated with a defined level of response – the benchmark response (BMR).

- For IQ, the BMCs were calculated for a BMR of a 1 point decrement in population mean IQ. On a population basis, a mean decrement in IQ of 1 point is associated with an added risk of mild mental retardation (MMR) of approximately 1 in 250 (385 in 100,000).
- The best estimate BMC associated with a 1 point decrement in mean IQ is 0.3 µg/dL, with a lower estimate BMC of 0.2 µg/dL and an upper estimate BMC of 1.4 µg/dL. The lower and upper estimates of the BMC reflect some, but not all, of the variability and uncertainty in the estimate of the slope of the blood lead concentration-IQ relationship.
- For SBP, the BMCs were calculated for a BMR of an increase in the population mean SBP of 1.3 mmHg. On a population basis, a mean increase in SBP of 1.3 mmHg is associated with a 1 in 20 (5,421 per 100,000) added risk of hypertension and a 1 in 2,000 (50 per 100,000) incremental risk of coronary heart disease mortality.
- The best estimate of the BMC for a 1.3 mmHg increase in mean SBP is 5 µg/dL for Caucasian males and 2.7 µg/dL for susceptible sub-populations. The lower estimate BMC is 1.6 µg/dL and the upper estimate BMC is 25 µg/dL. The lower and upper estimates of the BMC reflect some, but not all, of the variability and uncertainty in the estimate of the slope of the blood lead concentration-SBP relationship.

Presents an estimates of a blood lead distribution around the BMCs to derive riskspecific blood lead concentrations. A risk-specific blood lead concentration is the geometric mean blood lead concentration associated with a specified probability of population health effects. Data from national blood lead biomonitoring studies indicate that the current ratio of 95th percentile to geometric mean blood lead concentrations in the population is about three. Therefore, the risk-specific blood lead concentrations were derived by dividing the BMCs by a factor of three.

- The best estimate of the children's blood lead concentration where the mean leadassociated IQ decrement will be no more than 1 IQ point in 95% of the population is 0.1 µg/dL, with a lower and upper estimate of 0.1 to 0.5 µg/dL, respectively. At this geometric mean blood lead concentration, 5% the population would have an average IQ loss of 1 point or greater.
- The best estimate of the adult blood lead concentration where the mean leadassociated increase in SBP will be no more than 1.3 mmHg in 95% of the population is 1.7 µg/dL for Caucasian males and 0.9 µg/dL for susceptible sub-populations. The lower and upper estimates are 0.5 and 6.3 µg/dL, respectively. At this geometric mean blood lead concentration, 5% of the population would have an average increase in SBP of 1.3 mmHg or greater.
- The risk-specific blood lead concentrations derived in this report are intended to
 provide risk managers with a point of reference for making decisions about primary
 prevention of population health effects from lead exposure. The risk-specific blood
 lead concentrations are not intended to represent a threshold of effects; nor are they
 intended to represent an "acceptable" or "tolerable" risk. They simply quantify the
 blood lead concentrations associated with a specified level of population risk. The
 methods presented in this report may be used by risk assessors to calculate riskspecific blood lead concentrations associated with higher or lower population risk
 levels.

SECTION 7 • CANCER

Introduction

There are multiple lines of evidence, including human epidemiological studies, *in vivo* animal assays, and in vitro experiments, that support the hypothesis that lead exposure can induce cancer in humans. This report does not provide a comprehensive review and analysis of all of the data on this subject because recent comprehensive summaries of the evidence have been presented elsewhere (U.S. Department of Health and Human Services, 2003; IARC, 2006; US EPA, 2006). All of these reviews support the conclusion that the epidemiological evidence of an association between lead exposure and cancer in humans is limited but that there is sufficient evidence to conclude that lead is carcinogenic in animal experiments. Many of the available epidemiological studies suffer from methodological weaknesses, such as imprecise measurements of exposure, or not adequately controlling for confounding variables. On balance, the epidemiological literature does not provide strong evidence either for or against the conclusion that lead is a human carcinogen. In the absence of evidence of the contrary, it is assumed that animal carcinogens are also carcinogenic in humans.

This section of the report will:

Briefly summarize the available evidence from animal cancer bioassays and epidemiological studies of the carcinogenicity of lead and summarize the available evidence of the genotoxic properties of lead from multiple lines of inquiry. Lead is a confirmed animal carcinogen. However, the epidemiological evidence of an association between lead exposure and cancer in humans is limited. There is evidence that lead is genotoxic but insufficient evidence to establish whether lead is directly genotoxic or indirectly genotoxic. The results of assays to test the mutagenicity of lead are inconsistent. Review the categorization of lead as a human carcinogen, including evaluations by other health agencies, such as the International Agency for Research on Cancer (IARC) and the US National Toxicology Program (NTP) as well as apply Health Canada's criteria for categorization of carcinogens under the Canadian Environmental Protection Act (CEPA). Lead has been classified as "likely", "probably" or "reasonably anticipated" to be a human carcinogen by various health agencis. These categories are equivalent to a Group II carcinogen under Health Canada's CEPA categorization criteria. It is Health Canada's policy to quantify the potency of CEPA Group I and II carcinogens and to assess and manage exposure to these substances based on their most sensitive health endpoint.

Review the available information on plausible Modes of Action (MoA¹⁴) of lead induced carcinogenesis and discuss the implications for defining lead as a threshold or nonthreshold carcinogen. The MoA of carcinogenesis of lead is uncertain and there is insufficient evidence to identify a threshold for the carcinogenic effects of lead. Therefore, the following default assumptions apply: the MoA that is operative in animals is also relevant to humans and the cancer risks associated with lead exposure are characterized assuming that the dose-response curve is linear at low doses (i.e. no population threshold for effects).

Identify the critical studies for quantification of the cancer potency of lead. Two studies of renal tumors in mice, one from perinatal exposure (Waalkes *et al.*, 1995) and one from adult lifetime exposure (Waalkes *et al.*, 2004), were identified as candidate critical animal studies. No epidemiological study suitable for developing a quantitative estimate of the cancer potency of lead was identified.

¹⁴ Key events, processes, and obligatory steps, starting with the interaction of an agent with the target cell, through functional and anatomical changes, resulting in cancer or other adverse health effects.

Describe the methods and results of dose-response modeling of the candidate critical studies and the derivation of quantitative estimates of cancer potency from each. The carcinogenic potency of lead was estimated by using a multistage model to derive a Tumorigenic Dose 05 (TD₀₅). A TD₀₅ is the dose associated with a 5% increase in excess risk of exposure related pre-cancerous lesions or tumours. The most sensitive life-stage, sex, and endpoint was the excess risk of renal proliferative lesions among male mice exposed *in utero* and lactationaly only. The TD₀₅ for this endpoint, adjusted for potential differences in mouse to human kinetics, is 9.2 mg/kg/d. The cancer unit risk (slope factor) associated with this TD₀₅ is 5.46 x10⁻² (mg/kg/d)⁻¹. Based on this cancer unit risk, Health Canada's existing pTDI for lead of 0.0036 mg/kg/d is associated with an estimated Incremental Lifetime Cancer Risk (ILCR) of approximately 2 in 100,000.

Provide a quantitative and qualitative comparison of the relative potencies of the carcinogenic, cardiovascular, and neurotoxic effects of lead. The estimated blood lead concentration associated with a 1 in 100 added risk of coronary heart disease (CHD) mortality amongst susceptible subpopulations is 3 to 4 fold lower than the estimated blood lead concentration associated with a 1 in 100 excess risk of pre-cancerous renal lesions in a susceptible subpopulation.

- The estimated blood lead concentration associated with a 1 in 100 added risk of hypertension is 50 to 1,000 fold lower than the estimated blood lead concentration associated with a 1 in 100 excess risk of pre-cancerous renal lesions in a susceptible subpopulation.
- The estimated blood lead concentration associated with a 1 in 100 excess risk of MMR is 70 to 500 fold lower than the estimated blood lead concentration associated with a 1 in 100 excess risk of pre-cancerous renal lesions in a susceptible subpopulation.

• The weight of evidence supporting the carcinogenic effects of lead at environmentally relevant exposures is weaker than that for the developmental neurotoxicity and cardiovascular toxicity of lead.

7.1 EVIDENCE FOR THE CARCINOGENICITY AND GENOTOXICITY OF LEAD

7.1.1 Animal Cancer Assays

The available literature on lead as an animal carcinogen has recently been reviewed and summarized by the US National Toxicology Program (U.S. Department of Health and Human Services, 2003), the International Agency for Research on Cancer (IARC, 2006), the US Environmental Protection Agency (US EPA, 2006) and the US Agency for Toxic Substances and Disease Registry (US ATSDR, 2007). The conclusions of these reviews are that lead is a well established carcinogen in animal assays. There is evidence of an association between lead exposure and cancer or precancerous lesions in animals in renal, lung, and central nervous system (CNS) tissues. No animal study that was positive for stomach tumors was identified at the time of this report. The evidence for lung tumors is equivocal. The evidence for tumors of the CNS is positive, but limited, and the evidence for renal tumors is predominantly positive.

There has been a reported increased incidence in renal tumors of lead exposed animals in multiple species and strains, over multiple experiments, across varied dose levels, and with reported dose-response relationships in the expected direction. The spontaneous occurrence of renal carcinomas in rats is also very rare (IARC, 2006). At least 15 rat assays and at least two mice assays have reported significant associations between oral or subcutaneous exposure to soluble lead salts, most commonly lead acetate, and increased incidence of renal proliferative lesions, adenomas or carcinomas. Three studies, one each of mouse, hamster and rabbit, have reported no significant association between lead and renal tumors. The negative mouse and rabbit studies have methodological limitations and, although no

renal hyperplasia or tumors were observed in the renal tissue of the hamsters, kidney cells did show evidence of pre-neoplastic effects (pleomorphic cells and hypertrophic nuclei).

The weight of evidence supports the conclusion that soluble inorganic lead is a carcinogen in animal experiments and that the kidney is the most sensitive site of tumor occurrence in lead exposed rodents. There is also substantial evidence that lead is an effective promoter of renal tumors in rats.

The results from at least 50 published animal cancer bioassays for lead were reviewed for this report; however, no animal study that meets the currently recommended protocol of the US National Toxicology Program (NTP) for a chronic rodent cancer bioassay – two species (one each of rat and mouse), three dose groups and control, and starting with 50 animals per sex per dose group could be located (U.S. Department of Health and Human Services, 2006).

The limited power of cancer assays with less than 50 animals per dose group to detect a significant response should be recognized. The minimum detectable statistically significant tumor incidence, with a tumor incidence in controls of 0, is about 20% in experiments with 25 animals per dose group. The minimum detectable tumor incidence decreases by more than two-fold, to about 8%, if the number of animals per dose group is doubled (Eaton and Klaassen, 2001). This means that, at 25 animals per dose group, a tumor incidence of up to 19% could exist in the exposed animals, but it may not be detected as statistically significant. The minimum detectable tumor incidence will decrease from the values presented above as the incidence of background tumors in controls increases. Therefore, negative results from cancer assays with less than 50 animals per dose group should be viewed with caution because of their potential lack of power of to detect a significant response.

7.1.2 Epidemiological Studies

The available epidemiological literature on the association between lead exposure and cancer in humans has recently been reviewed and summarized by the International Agency for Research on Cancer (IARC, 2006), the US Environmental Protection Agency (US EPA, 2006) and the US Agency for Toxic Substances and Disease Registry (US ATSDR, 2007). The reader is referred to these sources for a more detailed presentation of the available literature on this subject.

There is limited epidemiological evidence that lead is a carcinogen. In general, epidemiological studies examined several common cancer endpoints which included lung, stomach, renal, brain and CNS, and all-site cancers¹⁵. A smaller number of studies examined cancer endpoints which included thyroid and endocrine, bladder, pancreatic, and prostate cancers, melanoma, lymphoma, and other cancers. This review focuses on the more commonly studied cancer endpoints. Overall, the weight of evidence is limited for an association between lead exposure and cancer in epidemiological studies for lung, stomach, renal, brain and CNS tissues, and all-site cancers. The evidence for lung, stomach and allsite cancer is positive but not conclusive. The evidence for renal and brain and CNS cancer is generally more equivocal.

The results from 29 cohort studies and 16 case control studies were critically reviewed. Cohort studies were most commonly completed for occupational exposures among lead smelter and battery factory workers. Population-based cohort studies were also completed, with accompanying lower dose environmental exposures, particularly amongst the National Health and Nutrition Examination Survey (NHANES) II and III cohorts from the United States. Cohort studies have also been completed on lead chromate pigment production workers,

¹⁵ all-site cancer refers to studies in which cancers were quantified in a variety of tissues, but the relative risk analysis was not conducted on a tissue-specific basis.

lead miners, workers involved in glass production (possible co-exposures to other metals, silica and asbestos), and newspaper printers (IARC, 2006). A very limited number of studies have examined workers exposed to organic lead, and specifically tetraethyl lead.

The majority of cohort studies were retrospective in design and quantification of exposure was most commonly completed through historical blood lead records. Blood lead records were often only single measurements and were not always available for all workers. Single blood lead measurements, as per the NHANES study design, are not optimal for quantifying relationships with long-term endpoints such as cancer because they represent only a single point in time during the often extended cancer latency period. Blood lead records were sometimes used to estimate more involved measures of exposure to lead, including cumulative blood lead index (CBLI), peak blood lead level, and number of years when at least one blood lead sample was obtained (Antilla, 1995; Englyst, 2001; Lundstrom, 2006). Certain studies also reconstructed lead dose through exposure surrogates such as a "job exposure matrix" or similar methods that involved the administration of questionnaires, estimation of time spent performing certain tasks, and estimation of lead exposure levels during those tasks (Rajamaran, 2006; van Wijngaarden, 2006; Rousseau, 2007). Exposure duration was also sometimes used as an exposure surrogate (Antilla, 1996; Cocco, 1997; Wong, 2000).

Confounding variables were present and unadjusted for in the majority of occupational cohort studies. The most significant confounding variables for lung cancer likely included smoking status and concurrent exposures to carcinogens such as arsenic. The majority of studies did not provide information on smoking status, and smoking likely confounded lung cancer findings to some extent. However, confounding from smoking is expected to be limited when relative risks between lung cancer and unadjusted exposure are greater than 1.4 (Siemiatycki, 1988). Furthermore, a positive association between lead exposure and lung cancer has been demonstrated in studies that did adjust for smoking, ethnic and, socioeconomic status (Siemiatycki, 1991) and among cohorts where it is unlikely that

significant exposure to other pulmonary carcinogens was correlated with lead exposure (Steenland, 1992). Thus, it is unlikely that all of the observed association between lead exposure and lung cancer can be attributed to residual confounding.

Concurrent exposures to substances including cadmium, copper, nickel, selenium, sulphur dioxide, dust, fumes and gases may also have occurred in many of the occupational studies involving lead smelter workers. Concurrent exposures to possible carcinogens including antimony, arsenic, cadmium, trivalent chromium, copper, manganese, nickel oxide and zinc selenite may have occurred in the occupational studies involving glass factory workers (Sankila, 1990). Concurrent exposure to cadmium may have confounded kidney cancer findings (Cocco, 1997). Furthermore, stomach cancer risk factors such as diet, ethnicity, *Helicobacter pylori* infection status and socioeconomic status were not always known and may have played a role in reported relative risks (for example, cases may not have been well-matched with controls in case control studies) (Siemiatycki, 1988; IARC, 2006).

The highest reported relative risks for lung, stomach and all-site cancer from numerous studies are plotted in Figure 33, Figure 34, and Figure 35, respectively. Data in these figures are from cohort and case control studies reviewed by IARC (2006) and additional studies published before 2008. The majority of the lung and stomach cancer data provide evidence for the positive association between lung and stomach cancers and lead exposure; however, many of these associations are not statistically significant (as their 95th percent confidence intervals fall below a relative risk of 1.0). The lung cancer association with lead exposure is more convincing than for stomach cancer, as results were statistically significant for seven positive studies (out of 23), as compared with only four positive studies (out of 17) for stomach cancer. The magnitude of the lung cancer associations are unlikely to be entirely explained by confounding by smoking (with the exception of Wong & Harris, 2000). Figure 35 demonstrates a suggestive association between all-site cancer and lead exposure, with three positive studies (out of 13) showing a statistically significant association.

Two meta-analyses that explore the association between lead and cancer in epidemiological studies provide supportive evidence for the association between lung, stomach and all-site cancer and lead exposure. The first meta-analysis, by Fu and Boffetta (1995), examined all available occupational studies. In a follow-up effort, Steenland and Boffetta (2000), conducted a similar analysis but limited the scope to only those studies that measured blood lead or environmental lead concentrations. The combined results from these two meta-analyses indicate that a quantitative analysis of the divergent individual epidemiological studies produces a significant positive association between occupational lead exposure and lung cancer and stomach cancer mortality. The magnitude of the associations further suggests that the associations are unlikely to be entirely explained by confounding.

Fu and Boffetta (1995) conducted a meta-analysis of available studies examining the association between occupational lead exposure and all-site (n=12), stomach (10), lung (12), kidney (5) and bladder cancer (5). A fixed effect model was used for all sites except lung as significant heterogeneity was observed among the lung studies and a random effect model was therefore used for this cancer endpoint. The relative risks (RR) reported in the meta-analysis were; all-site: 1.11 (1.05-1.17); stomach: 1.33 (1.18-1.49); lung: 1.29 (1.10-1.50); kidney: 1.19 (0.96-1.48); bladder: 1.41 (1.16-1.71). A secondary meta-analysis restricted to studies in industries with heavy lead exposure, smelting and battery works, yielded stomach and lung cancer mortality risks that were approximately 20% higher than those calculated from the entire collection of studies. The greater risks associated with qualitatively higher occupational lead exposures is evidence of a dose-response trend in the expected direction. The authors note that the small number of published results for renal and bladder cancers relative to lung and stomach cancers is suggestive of a publication bias for these sites.

Steenland and Boffetta (2000) conducted a meta-analysis of occupational studies of the association between lead exposure and cancer that also included measurements of blood or

environmental lead. A total of eight studies, seven cohort and one nested case-control, were identified that met these criteria. None of the studies included in this meta-analysis controlled for smoking or diet. All of the studies included in this meta-analysis had relatively high lead exposures; the average blood lead of the study cohorts ranged from 26 µg/dL to 80 µg/dL. The authors concluded that the results of the meta-analysis suggested only weak evidence of an association between occupational lead exposure and cancer, with the lung, stomach and gliomas as the sites of cancer with the most supporting evidence. There was significant heterogeneity in results among the eight studies that included lung cancer as an endpoint. A random effects meta-analysis of these studies yielded a RR of 1.30 (95% CI: 1.15-1.46). A fixed effects meta-analysis, excluding Lundstrom (1997) on the grounds that the unusually high RR reported from this study may be attributed to concomitant arsenic exposure, yielded a RR of 1.14 (1.04-1.25). A fixed effects meta-analysis of eight stomach cancer studies yielded a RR of 1.34 (1.14-1.57). According to Siemiatycki et al. (1988), relative risks greater than 1.2 for stomach cancer may be approaching significance despite the presence of any confounding covariates. The meta-analysis of other cancer endpoints yielded non-significant associations; a fixed effects meta-analysis of seven brain cancer studies yielded a RR of 1.06 (0.80-1.40) and a fixed effects meta-analysis of seven renal cancer studies yielded a RR of 1.01 (0.72-1.42)

The weight of evidence from the review of 29 cohort and 16 case control studies and the meta-analyses was evaluated to determine the strength of the evidence of an association between human lead exposure and the development of cancer. The strongest evidence supports an association between lead exposure and lung, stomach, and all-site cancers. Data for kidney and brain and CNS cancers were more equivocal, and only a handful of studies (n=3 for kidney and n=4 for brain & CNS) were able to show a statistically significant positive association.

Kidney cancer is less common than other cancers; this diminishes the power to detect a significant association with lead exposure, to identify an exposure-response relationship, and

to control for covariates (U.S. Department of Health and Human Services 2003). Kidney, brain and CNS cancer endpoints were thus excluded from further analysis.

The following conclusions are based on the Bradford Hill criteria for causality:

- Strength of Association: There is a stronger positive association between lead exposure and lung, stomach and all-site cancers than for kidney, brain and CNS cancers; these associations are also supported by data with a higher statistical significance.
- Temporality: Most of the epidemiological studies were retrospective by design, and the temporal relationship between exposure and outcome was often difficult to determine.
- Consistency: More consistent associations are observed between lead exposure and lung, stomach and all-site cancers than for kidney and brain and CNS cancers. In addition to occupational data, lung and all-site cancer associations are supported by more environmental, population-based data than other cancers.
- Theoretical Plausibility: The mode of action of lead induced carcinogenesis is uncertain.
- Dose-Response Relationship: Few epidemiological studies demonstrate clear doseresponse relationships between lead exposure and cancer endpoints. However, those that did were more often those for lung, stomach and all-site cancers.
- Experimental Evidence: There is discordance between conclusions based on animal studies and those based on epidemiological studies. Specifically, the animal data suggests the strongest association between lead exposure in renal tissues, with weaker associations for lung and CNS tissues.

Overall, the weight of evidence for the epidemiological association between lead exposure and cancer is limited. The strongest evidence for causality exists between lead exposure and lung, stomach and all-site cancers. For this reason, decision-making criteria were then applied to assess the quality of individual studies for lung, stomach and all-site cancers in order to select candidate studies from which to derive a quantitative estimate of the carcinogenic potency of lead. Decision-making criteria included: a) strength of association and statistical significance (searching for a strong, positive and statistically significant association between lead exposure and a particular cancer endpoint); b) presence/absence of a dose-response relationship; c) reliability of exposure data (whether the study measured blood lead levels in individual participants, and how often); d) representative nature of results (whether exposures were representative of those for the general population); e) control of possible confounding (whether confounding from covariates was controlled for).

FOR ENVIRONMENTAL LEAD EXPOSURE IN CANADA



Figure 33. Highest Reported Relative Risks for Lung Cancer

Notes:

- Studies include cohort and case control studies reviewed by IARC (2006) and additional relevant studies prior to 2008.
- Error bars represent the 95th percent confidence intervals.
- During selection of highest reported relative risk: a) data for both sexes was favored over data for a single sex; b) highest reported statistically significant relative risk, when available, was selected over relative risk that was not statistically significant; c) relative risk with associated 95th percent confidence interval, when available, was selected over relative risk with no associated confidence interval.

FOR ENVIRONMENTAL LEAD EXPOSURE IN CANADA

Stomach Cancer 8 12 19.1 21.6; Cls 7 3.2 99.9 6 **Relative Risk** 5 4 3 2 0 Gerhardsson et al. 1995 Fanning 1988 Nong & Harris 2000 (cohort) Nong & Harris 2000 (case control) Cocco et al. 1996 Cocco et al. 1998 Jemal et al. 2002 Cocco et al. 1997 Englyst et al. 2001 undstrom et al. 1997 Sankila et al. 1990 Steenland 1992 Gerhardsson et al. 1986 Cordioli et al. 1987 Cocco et al. 1994a Cocco et al. 1994b Michaels et al. 1991 Cocco et al. 1999 Siemiatycki 1991 Anttila et al. 1995 -am et al. 2007 Rousseau et al. 2007 Sheffet et al. 1982 ⁼ayerweather et al. 1997

Figure 34. Highest Reported Relative Risks for Stomach Cancer

Notes:

- Studies include cohort and case control studies reviewed by IARC (2006) and additional relevant studies conducted prior to 2008.
- Error bars represent the 95th percent confidence intervals (CI).
- During selection of highest reported relative risk (RR): a) data for both sexes was favored over data for a single sex; b) highest reported statistically significant relative risk, when available, was selected over relative risk that was not statistically significant; c) relative risk with associated 95th percent confidence interval, when available, was selected over relative risk with no associated confidence interval.
- Upper 95th CI for Cocco et al. (1996) is 12; for Jemal et al. (2002) it is 19.1. The highest RR for Siemiatycki (1991) is 21.6, with CIs of 3.2-99.9.



Figure 35. Highest Reported Relative Risks for All-site Cancer.

Notes:

- Studies include cohort and case control studies reviewed by IARC (2006) and additional relevant studies conducted until 2008.
- Error bars represent the 95th percent confidence intervals (CI).
- During selection of highest reported relative risk (RR): a) data for both sexes was favored over data for a single sex; b) highest reported statistically significant relative risk, when available, was selected over relative risk that was not statistically significant; c) relative risk with associated 95th percent confidence interval, when available, was selected over relative risk with no associated confidence interval.

7.2 CATEGORIZATION OF LEAD AS CARCINOGEN

Several health agencies have recently conducted a weight of evidence review and categorization of the carcinogenicity of lead. The unanimous conclusion of these reviews is that lead is a probable (or equivalent adjective) human carcinogen based on conclusive evidence of carcinogenicity in animal bioassays and limited epidemiological evidence. In 2006 the International Agency for Research on Cancer (IARC) concluded that inorganic lead compounds are probably carcinogenic to humans (Group 2A), on the basis that there was limited evidence in humans, but sufficient evidence in experimental animals for the carcinogenicity of inorganic lead compounds,. The US EPA (2006) concluded that lead would probably be classified as 'likely to be carcinogenic to humans' if it were assessed according to the 2005 EPA *Guidelines for Carcinogen Risk Assessment*. The US National Toxicology Program (NTP) of the Department of Health and Human Services concluded in its 11th Report on Carcinogens (ROC) that lead and lead compounds are reasonably anticipated to be human carcinogens (U.S. Department of Health and Human Services, 2004).

Health Canada's criteria for categorizing carcinogens under the Canadian Environmental Protection Act (CEPA) are defined in Appendix B of the *Canadian Environmental Protection Act Human Health Risk Assessment for Priority Substances* (Health Canada, 1994). These criteria are based on those of IARC. Health Canada's criteria for a Group II carcinogen are:

"Group II — Data from epidemiological studies are inadequate to assess carcinogenicity either because there are few pertinent investigations or because chance, bias or confounding cannot be excluded as a possible explanation for the results. However, there is sufficient evidence of carcinogenicity in animal species (i.e., there is an increased incidence of malignant tumours in multiple species or strains, in multiple experiments with different routes of exposure or dose levels,

or the incidence, site or type of tumour or age of onset is unusual). Confidence in the sufficiency of the data from animal studies is increased when there is evidence of a dose-response relationship, supporting results from in vitro studies or a number of limited carcinogenicity bioassays, evidence of structure-activity relationships, genotoxic effects and/or supporting data on a mechanism of carcinogenicity which is operative in humans and animal species. Exceptionally, a compound for which the evidence of carcinogenicity is limited but for which there is a strong supporting dataset (on genotoxicity, for example) which indicates that the compound is likely to be carcinogenic would be included in this category." (Health Canada, 1994)

Inorganic lead meets the criteria for a Health Canada Group II carcinogen. The data from epidemiological studies is suggestive, but the strength of this evidence is generally limited by methodological shortcomings, such as imprecise exposure measurements or inadequate control of potentially confounding variables. There is strong evidence of carcinogenicity in animal species: an exposure related increase in malignant tumours has been reported in multiple species via multiple routes of exposure and in some cases, such as gliomas and renal carcinomas, the tumour site and type is unusual. A dose-response trend in the expected direction has been reported for some tumour sites in some studies; however not all studies used multiple exposure groups. There is evidence of genotoxic effects associated with lead exposure. There are several plausible Modes of Action and associated mechanisms explaining the observed renal carcinogenicity in animals and these mechanisms are also generally relevant to humans (see below for more discussion). Therefore, inorganic lead is classified as a Group II carcinogen via Health Canada's CEPA carcinogen classification scheme.

Health Canada typically derives quantitative estimates of cancer potency for assessing environmental exposure risks to substances which are categorized as Group I or II carcinogens under the CEPA scheme for classification of carcinogenicity; quantitative estimates for CEPA Group I or II carcinogens are required in the Protocol for the regulation of substances under CEPA (Health Canada, 1994; Health Canada, 1996) and the development of Canadian Soil Quality Guidelines (CCME, 2006) and Canadian Drinking Water Quality Guidelines (Health Canada, 1995). Environmental quality criteria or other quantitative risk estimates are then derived on the most sensitive health endpoint (Health Canada, 1995; CCME, 2006).

Mode of Action of Lead Related Carcinogenesis

The two key questions that need to be addressed with respect to the mode of action (MoA) of lead related carcinogenesis are:

- Whether the putative MoA of carcinogenesis observed in animal cancer bioassays is relevant in humans; and,
- Whether there is a threshold below which the MoA (or MoAs) that induce lead related cancer are inoperative.

These two questions are addressed sequentially below.

Mode of Action and Human Relevance

There are several plausible MoAs that could give rise to cancer as a result of lead exposure, with varying degrees of evidence supporting each. However, none of the proposed modes of action are yet supported by strong evidence of the underlying mechanism(s)¹⁶ of action.

The possible MoAs for lead to cause or modify carcinogenesis are reviewed here because the recently available reviews (IARC, 2006; US EPA, 2006; US ATSDR, 2007) do not include a systematic review of the underlying MoAs. Further, the relative

¹⁶ Detailed understanding at biochemical and molecular level.

weight of evidence for various plausible MoAs is important in determining whether the carcinogenesis observed in animal cancer bioassays is relevant to humans and whether lead should be assessed as a threshold or non-threshold carcinogen.

Silbergeld (2003) and Silbergeld *et al.* (2000) provide a thorough discussion and presentation of supporting evidence for the proposed MoAs for lead and cancer. A literature search revealed that, since Silbergeld (2003), there has only been one publication relevant to the subject (Waalkes *et al.*, 2004). Therefore, the summary below draws primarily on the information presented in Silbergeld (2003) and Silbergeld *et al.* (2000) and readers are referred to these primary sources for a more detailed discussion.

There has been a great deal of recent work on establishing the Human Relevance Framework for evaluating the relevance of animal tumor data for human cancer risk assessment (Meek *et al.*, 2003; Cohen *et al.*, 2004; Boobis *et al.*, 2006). However, the Framework is not universally accepted (Guyton *et al.*, 2008) and the difficulty of its application in cases where there are multiple plausible MoAs or the MoA is dependant or modified by other exposures has been noted (Caldwell *et al.*, 2008). Nonetheless, the Framework does offer a systematic conceptual framework for evaluating the relevance of evidence from animal carcinogenicity assays to human cancer risks.

Concordance between human and animal studies in the site of tumorgenesis is a fundamental premise upon which the Framework is based. There is limited concordance between animal studies and epidemiological data on the site of lead tumorgenesis. The greatest evidence from the animal literature is for the association between exposure to lead acetate or lead subacetate and renal adenomas and carcinomas in rodents. This tumor site been studied in epidemiological investigations, but the results are equivocal. As with the existing epidemiological studies of lead and cancer in general, the epidemiological studies of lead and renal cancers suffers from

methodological issues such as imprecise exposure measurement and potential confounding. Nonetheless, there are some epidemiological studies showing a significant positive association between lead exposure and renal cancers in humans (Siemiatycki, 1991; Steenland, 1992; Pesch, 2000). Therefore, there is a degree of concordance between animal and human studies on renal carcinogenesis. The Framework also specifies that the likelihood of congruence between target organs in test animals and humans should be evaluated based on the MoA (Cohen *et al.*, 2004).

The first question within the Human Relevance Framework is whether there is sufficient weight of evidence to establish the MoA in animals. The available evidence for possible MoAs for lead is summarized below. Based on a review of the current literature there is suggestive, but insufficient, evidence to clearly establish a MoA for the most frequently reported tumor sites in animal studies (renal). The Framework recommends that where there is insufficient evidence to establish a MoA in humans the underlying animal MoA should be assumed valid in humans.

A full analysis of the lead and cancer data based on the Human Relevance Framework is not possible because:

- There is limited concordance between animal and human studies in the site of tumor occurrence
- There is insufficient evidence to establish a MoA in animals

For these reasons, the entire body of evidence supporting lead as a human carcinogen is not as strong as the evidence for other critical endpoints, such as neurotoxicity and cardiovascular effects, for which there is a strong degree of concordance of evidence from *in vitro*, animal and human studies.

The evidence for possible MoAs for lead and cancer are summarized below and are categorized as genotoxic and non-genotoxic MoAs. Genotoxic carcinogens directly or

indirectly cause damage to DNA, including adduct formation, oxidative alteration, or strand breakage. Non-genotoxic carcinogens do not directly alter DNA, but cause cancer by promoting carcinogenesis initiated by genotoxic agents or spontaneous DNA damage by enhancing cell division or inhibiting apoptosis. Non-genotoxic MoAs include dysregulation of gene expression, dysregulation of transcription, dysregulation of signal transduction, and impairment of DNA repair mechanisms. Health Canada's default approach is to assess genotoxic carcinogens as non-threshold carcinogens (Health Canada, 1994; Health Canada, 1996).

Genotoxic MoAs

There is sufficient evidence that lead is genotoxic; however, it is unclear whether lead is directly or indirectly genotoxic, and the genotoxicity of lead at environmentally relevant doses is uncertain. Most of the available *in vitro* assays tested lead compound concentrations in the milimolar and micromolar concentration ranges, whereas environmentally relevant doses are in the nanomolar range¹⁷. There is evidence supporting two mechanisms of action of indirect genotoxic effects of lead: 1) the production of reactive oxygen species (ROS) and 2) the interference with DNA repair. The evidence of genotoxicity and mutagenicity of lead from human and animal *in vivo* tests as well as *in vitro* experiments is varies depending on the lead compound tested, dose or concentration, experimental procedure and test endpoint. The available evidence of the genotoxicity and mutagenicity of lead is reviewed in detail in Roy *et al.* (1992), IARC (2006), US EPA (2006), US ATSDR (2007), U.S. Department of Health and Human Services (2003), and Hartwig (1994; 1995). Very brief summaries are presented below.

¹⁷ 1 nM serum Pb is approximately equal to 10 μ g/dL whole blood Pb, assuming a serum:RBC partitioning of 0.24% as reported by Manton et al. 2001.

The divalent lead cation (Pb²⁺) appears to be only weakly mutagenic and possibly only at cytotoxic doses, but Pb²⁺ might act as a co-mutagen at lower concentrations. There is evidence of mutagenic effects of lead nitrite, lead chloride and lead sulfide in Chinese Hamster Ovary (CHO) cells at doses less than the reported cytotoxic LD₅₀ (Zelikoff *et al.*, 1988; Ariza *et al.*, 1998). The LOAEL for *in vitro* mutagenic effects of lead chloride reported by Ariza *et al.* (1998) was 0.1 μ M (~2 μ g Pb/dL serum). Roy and Rossman (1992) also identified an *in vitro* NOAEL for the mutagenic effects of lead acetate and lead nitrate in a transgenic CHO cell line (G12). Pre-exposure of the same cell line to a non-mutagenic and slightly cytotoxic concentration of lead acetate (0.4 mM) for 24 hours increased the cytotoxicity and mutagenicity of *N*-methyl-*N*'-nitro-*N*-nitroguanidine and UVC light (Roy and Rossman, 1992). A possible mechanism of comutagenicity of lead is the inhibition of nucleotide excision repair (Hartwig *et al.*, 1990).

Human In Vivo

There is evidence of an association between genotoxicity (sister-chromatid exchange, micronuclei, and DNA-protein crosslinks) and occupational lead exposure (Rajah and Ahuja, 1995; Vaglenov *et al.*, 1997; Bilban, 1998; Donmez *et al.*, 1998; Ye *et al.*, 1999; Restrepo *et al.*, 2000; Vaglenov *et al.*, 2001). These recent studies do a better job at controlling for potential confounding variables such as smoking, alcohol consumption, and occupational exposure to other genotoxins than do the equivocal results from earlier occupational studies. Genotoxic effects have been reported in association with occupational blood lead concentrations as low as about 15 μ g/dL (Donmez *et al.*, 1998; Ye *et al.*, 1998; Ye *et al.*, 1998). The available studies of genotoxic effects in environmentally exposed humans report conflicting results.

Animal In Vivo

The evidence of genotoxicity of lead from animal studies is varied, depending on the route of exposure, lead compound tested, dose and test endpoint. Lead exposure in animals has been associated with DNA strand breakage, sister-chromatid exchange (SCE), micronucleus formation, aneuploidy, and increased frequencies of chromosomal aberrations. However, these positive results have generally been associated with relatively high lead doses and blood lead data are not available for most studies.

Plant In Vivo

Most *in vivo* plant studies report an association between lead exposure and clastogenic effects.

In Vitro Eukaryotic

There have been variable responses for DNA strand breakage, sister chromatin exchange, chromosomal aberrations, and mutagenicity of lead compounds in mammalian cell cultures, with different results for different lead compounds, test concentrations, and endpoints. Micronucleus formation has been associated with low lead exposure. The LOAEL for *in vitro* mutagenic effects of lead chloride reported by Ariza *et al.* (1998) was 0.1 µM, approximately equivalent to 2 µg Pb/dL serum. If it is assumed that serum lead is 0.24% of whole blood (Manton *et al.*, 2001), this would be equivalent to a whole blood concentration of approximately 860 µg/dL.

In Vitro Prokaryotic

The evidence of mutagenicity of lead from bacterial assays is variable and dependent on the lead compounds tested. The results for lead acetate and lead chloride have been negative, while positive results were reported for lead bromide and lead chromate. It is possible that the positive results from the latter compounds may be attributed to the anions, rather than the lead.

Indirect Genotoxicity

Lead may not be directly genotoxic but may cause genetic damage through several plausible indirect mechanisms such as; inhibition of DNA synthesis and repair, oxidative damage, interaction with DNA binding proteins and tumor-suppressor proteins. Lead exposure may result in the formation of free radicals which are capable of causing oxidative damage to DNA. Suspected mechanisms include the depletion of antioxidants and consequent reduction in cellular defense against insult from ROS induced by other agents, catalysis of Fenton-type reactions (whereby hydroxyl and singlet oxygen free radicals are generated from hydrogen peroxide) (Roy and Rossman, 1992), and accumulation of aminolevulinic acid (ALA). ALA can in turn generate free radicals that are capable of causing oxidative damage to DNA (Yusof *et al.*, 1999).

Non-genotoxic MoAs

There is no strong direct evidence supporting any one non-genotoxic or epigenetic MoA for lead. However, the lack of clear evidence of a genotoxic MoA and the strong evidence that lead is at least an animal carcinogen lends support to the conclusion that lead is likely an epigenetic carcinogen. Lead may result in epigenetic effects directly, or may act as a promoter by facilitating the effects of other genotoxic agents.

Chronic lead exposure induces nephropathy at high doses. Tumors may arise as a secondary effect due to cellular responses to nephropathy. However, recent animal

studies have demonstrated that renal tumors are induced at lead exposures that do not result in chronic nephropathy (Waalkes *et al.*, 1995).

Lead has been shown to have a mitogenic effect in both renal and hepatic tissues. The mitogenic effect of lead on hepatic tissue has been determined to be proliferative mitogenesis and, in contrast to regenerative mitogenesis, it is thought that proliferative mitogenesis does not contribute to hepatocarcinogenesis. Mitogenesis, however, remains a plausible mechanism in the causal pathway for renal cancers.

Some renal carcinogens act through binding to α 2u-globulin - a MoA that is specific to the male rat. Carcinogenicity by α -2u-globulin binding is characterized by excessive accumulation of protein droplets in proximal tubule epithelial cells. The ligand-bound proteins are resistant to hydrolysis and their accumulation leads to a cytotoxic response which, in turn, leads to compensatory renal cell proliferation. However, as lead induces renal cancers in both male and female rats (van Esch *et al.*, 1962) and mice (Waalkes *et al.*, 1995) the MoA of renal tumors is not limited to α 2u-globulin binding.

Lead can substitute for Zn in Zn-binding proteins, including the DNA binding proteins histones, protamines and transcription regulators Sp1 and TFIIA (Silbergeld, 2003). Lead binding to Zn-binding proteins can also alter their conformational structure and function, as is evident with lead's well known inhibition of the Zn-binding enzyme δ -ALAD. Zn-binding proteins have varied functional roles, including gene expression, gene transcription and DNA repair. Lead substitution in these proteins would alter their structure in ways that may alter their function and altered gene function or cellular signaling may result in progression of carcinogenesis. Evidence of lead altering expression of oncogenes and tumor suppressor genes is reviewed in Silbergeld (2003).

Lead induced renal tumors and hyperplasia in mice have been shown to contain an over-expression of cyclin D1(Waalkes *et al.*, 2004). Cyclin D1 is involved in the regulation of the cell cycle, is an oncogene, and is associated with various cancers including human renal cell carcinoma (Lin *et al.*, 1998; Hedberg *et al.*, 1999).

Fowler *et al.* (1994) and Fowler (1998) proposed a MoA for renal cancer whereby soluble lead-binding proteins mediate intranuclear movement and chromatin binding of lead in renal target cell nuclei. It was suggested that the chromatin binding could alter gene expression resulting in tumorgenesis. However, subsequent research by Waalkes *et al.* (1995, 2004) demonstrated that a reduction in lead bound to proteins (metallothionein) increased the risk of renal tumors - opposite to the expected direction of effect predicted by Fowler.

Lead may also behave as a co-carcinogen or promoter, and four of six animal studies investigating the carcinogenic potential of interaction between lead and other carcinogens showed a positive response (reviewed in (Silbergeld, 2003) and (U.S. Department of Health and Human Services, 2003): Shakerin et al. (1965) reported an increased incidence of hepatic and renal tumors in rats exposed to dietary lead and 2acetylaminofluorene (2-AAF) whereas Hass et al. (1967) and Oyasu et al. (1970) failed to reproduce these results. Dietary lead subacetate increased the incidence and size of renal tumors induced by dietary exposure to *N*-ethyl-*N*-hydroxyethylnitrosamine (EHEN) in rats (Hiasa et al 1983). Tanner et al. (1984) reported an increased incidence of renal tumors in N-(4'-fluoro-4-biphenyl)acetamide (FBPA) and lead acetate exposed rats when compared to rats exposed to either lead acetate or FBPA. Renal tumor incidences were not increased by co-administration of lead acetate and sodium nitrite and ethyl urea (Koller et al., 1985). Two additional studies reported an increase in the incidence of lead related renal tumors in rats as a result of co-exposure to calcium (Bogden et al., 1991; Bogden et al., 1995). There is epidemiological evidence that lead is associated with increased risk of lung cancer among smokers (Lustberg and

Silbergeld, 2002) and evidence that co-exposure to lead and arsenic in occupational settings is associated with increased risk of lung cancer (Englyst *et al.*, 2001). *In vivo* evidence shows that combined exposure to lead oxide and benzo(*a*)pyrene (BaP) caused lung tumors in hamsters where exposure to similar doses of either chemical in isolation did not induce lung tumors (Kobayashi and Okamoto, 1974). Lead also increased the mutagenicity of *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (NMNG) and UV light *in vitro (Roy and Rossman, 1992)*.

There is evidence that lead may interfere with repair of UV-induced DNA damage (Hartwig *et al.*, 1990).

Lead exposure has been associated with reduced DNA methylation which, in turn, could affect the expression and regulation of genes that are important in the etiology of cancer. Relatively low, environmentally relevant, early life lead exposure in mice and monkeys has been shown to alter DNA methylation and gene expression and regulation later in life. Altered DNA methylation has been investigated as a possible mechanism for the observed association between early life lead exposure and the late life development of brain pathologies that are characteristic of Alzheimer's Disease: Rodent studies showed that lead exposure during brain development predetermined the expression and regulation of ß-amyloid precursor protein (APP) and the production of ß-amyloid (Aß) peptides latter in life (Basha et al., 2005). The expression of Alzheimer's Disease related genes, APP and β -site APP cleaving enzyme 1 (BACE1), and their transcriptional regulator (Sp1) were elevated in aged monkeys exposed to lead only as infants (Wu et al., 2008). These latent genetic effects were associated with a decrease in DNA methyltransferase 1 (DMT1) activity; DMT1 is directly proportional to the abundance of methyl groups on CpG dinucleotides in the DNA. In vitro experiments have also shown that low levels of lead exposure to mouse primary cells can reduce DMT1 activity (Wu et al., 2008). At the time of this report no publications on the possible role of lead in altering the methylation patterns and
associated expression and regulation of genes that are specifically implicated in the development of cancer had been located in the existing literature.

In summary, there are several plausible and competing theoretical MoAs for leadassociated carcinogenesis. Some of these MoAs are genotoxic (either directly and indirectly by oxidative damage) while others are epigenetic. However, there is insufficient evidence to identify any single MoA as the most likely or definitive MoA and carry it forward in the analysis prescribed by the Human Relevance Framework. The default assumption, therefore, is that the MoA that is operative in the development of cancer in animals is also relevant to humans.

7.3 THRESHOLD FOR CARCINOGENIC EFFECTS OF LEAD

Health Canada's default assumption is that there is some probability of cancer at every level of exposure to genotoxic carcinogens, and, therefore, these chemcals are assessed as non-threshold substances (Health Canada, 1994; Health Canada, 1996). As discussed above, there is insufficient evidence to clearly establish lead as a genotoxic or non-genotoxic carcinogen. Furthermore, even if it were possible to make the case that lead is a non-genotoxic carcinogen and acts via a threshold mechanism of action, a threshold for the carcinogenic effects of lead would have to be identified. There is insufficient evidence to establish a clear threshold for the carcinogenic effects of lead. In this context the importance of establishing lead as a genotoxic or non-genotoxic carcinogen is reduced. For the purposes of the quantification and assessment of the cancer potency of lead, it will therefore be assumed that there is no threshold.

7.4 CANDIDATE CRITICAL STUDIES FOR CANCER

A critical study is defined as a high quality study that reports a statistically significant increase in incidence of exposure related tumours at a site that is supported by the weight of evidence and for which the MoA is either not understood or is confirmed to be relevant to humans. Criteria for selection of a critical study may vary according to the type of evidence being considered (animal bioassay or epidemiological), the cancer site, and the quality and quantity of available studies from which to select. Justification for the identified candidate critical animal studies used in this analysis is provided below. It was not possible to identify an adequate epidemiological study for the development of a quantitative estimate of cancer potency. Candidate critical epidemiological studies were reviewed but ultimately rejected, and are described below to support the final determination. Candidate critical studies were thus nominated from animal studies only, and the candidate critical study that produced the highest estimate of cancer potency was selected as the critical study for the cancer endpoint. These results are not surprising given inorganic lead's classification as a Health Canada Group II carcinogen in recognition that the strength of the epidemiological evidence is limited.

7.4.1 Candidate Critical Animal Studies

Two candidate critical animal studies were identified (Waalkes *et al.*, 1995; Waalkes *et al.*, 2004) and are described below: These two critical studies were chosen because each of these studies examines the tumor response as a result of lead exposure at different life stages. Although these are not parallel studies (different strains were used and different dose regimes were used), the fact that these 2 studies were completed in the same lab under relatively comparable experimental conditions and design presented an opportunity to develop quantitative estimates of the renal cancer potency of lead for both perinatal (*in utero* and lactational) and adult lifetime exposure to lead.

Many of the animal cancer assays for lead were performed 20 years or more ago and suffer from incomplete reporting and/or methodological issues. None of the available animal assays meet the current 'gold standard' in animal cancer bioassays which is a chronic two year rodent study with rats (typically F344) and mice (typically B6C3F1) of both sexes, 50 animals per dose group, and two or three dose groups in addition to a control group. Because of the increased cancer risk associated with excessive weight gain, controlled access to food is also currently recommended.

No study that met all of the criteria of the gold standard in cancer bioassays was identified at the time of this report. The mouse study by Waalkes *et al.* (1995) is well designed and documented and, of the published studies reviewed, most closely meets the above criteria. However, the Waalkes *et al.* (1995) study deviates from these standards in a couple of important aspects: 1) the exposure was not lifetime, but perinatal only (transplacental and translactational); 2) dose groups were 23-25 animals, rather than the prescribed 50; and 3) results are reported for renal tissue only. The authors of the paper state that analysis for other tissues will be published elsewhere but, when contacted, the lead author Dr. Waalkes indicated that the results from the extra renal tissues had not been published. Dr Waalkes was also unable to provide the unpublished study data, but provided assurances that there were no positive results for other cancer sites (Waalkes 2008, *pers. com.)*. Despite these considerations, the Waalkes *et al.* (1995) study is of sufficient quality to derive a quantitative estimate of the renal cancer potency of perinatal lead.

Waalkes et al. 1995

Female C57BL/6NCr mice were bred with C3H/HeN males and were administered 0, 500, 750, and 1000 ppm lead acetate via drinking water *ad libitum* starting at gestational day 12. Exposure was continued until 4 weeks postpartum. Assuming the maternal mice weighed 25 g and consumed 5 ml of water per day, the maternal lead

doses are equivalent to 0, 100, 150 and 200 mg/kg/d¹⁸. Progeny (B6C3F₁ mice) were then weaned and observed for up to 112 weeks. Litter size and body weight and survival of progeny were not affected at any of the exposure doses. Necropsies were performed on all animals. Incidence of renal proliferative lesions (RPL), including atypical tubular hyperplasia and tumors, in gestationally and lactationally exposed mice were reported as: control, 4%; 500 ppm, 16%; 750 ppm, 24%; 1000 ppm, 48%. The number of mice assessed in each exposure group ranged from 23-25. Renal tumors developed in the absence of evidence of significant concurrent lead-induced chronic nephrotoxicity. In a parallel experiment, mice with the same perinatal lead exposure were also exposed to 500 ppm of the renal tumor promoter barbital sodium (BB) in drinking water from weaning onward. Postnatal BB exposure had no significant effect on the incidence of RPL.

Waalkes et al. (2004)

Waalkes *et al.* (2004) conducted a chronic study of the renal effects of lead acetate in the drinking water of male wild-type (WT) and metallothionein –I/II knockout mice. The results from the WT mice can be used to quantify the renal cancer risk for adult only "lifetime" lead exposure. Comparisons to the perinatal mouse cancer assay (Waalkes *et al.*, 1995) should be done with caution because this assay used a different strain of mice, males only, and a higher dose regime. Nonetheless, the Waalkes *et al.* (2004) study was judged to be the highest quality study available with which to quantify the renal cancer risks from adult lifetime lead exposure. Starting at 8 weeks, male mice were exposed to lead acetate in drinking water (*ad libitum*) at concentration of 0 (control), 1,000 ppm, 2,000 ppm, or 4,000 ppm lead. Assuming the adult mice weighed 25 g and consumed 5 ml of water per day, the lead doses are equivalent to 0, 200,

¹⁸ The authors did not measure blood Pb concentrations in the exposed mice. As a point of comparison, Fox et al. (2008) reported that exposure of rat dams to 100 ppm Pb in drinking water produced peak blood Pb of approximately 45 μ g/dL in progeny at post natal day 10.

400, and 800 mg/kg/d. Mice were observed up to 112 weeks of age. Survival of WT mice was significantly reduced at the highest exposure (4,000 ppm lead). Significant depression in body weight of WT mice occurred in the 2,000 ppm (7-9% less than control) and 4,000 ppm (12-14% less than control) exposure groups. Necropsies were performed on all animals. Incidence of renal proliferative lesions (RPL), including atypical tubular hyperplasia and tumors, in chronically adult exposed WT mice were reported as: control, 0%; 1,000 ppm, 4%; 2,000 ppm, 12%; 4,000 ppm, 21%. Renal adenoma and cystic hyperplasia are considered precursor lesions to renal cell carcinoma. Pre-neoplastic epithelial cell hyperplasia is often observed in association with renal cancers in rodents (Waalkes *et al.*, 2004). Chronic adult lead exposure was not associated with a significant increase in tumors of any other tissues that were pathologically examined, including lung. Brain tissue was not examined. The incidence of hepatic tumors decreased with increasing lead dose, with a stronger protective effect reported for the metallothionein-null mice.

The chronic rat and dog feeding studies by Azar *et al.* (1972) were also considered as a candidate critical animal studies. However, these data do not appear to have been published in a peer reviewed paper, the methods of pathological examination and classification are not described, and the incidence of proliferative lesions is not reported. Additionally, the variance in response was not reported and, therefore, it was not possible to conduct dose-response modeling with the published data. Renal tumors were reported in male rats (strain not identified) at dietary lead exposure doses as low as 548 ppm. Slight cytomegaly was found in the proximal convoluted tubule of some of the male dogs treated at 500 ppm dietary lead, but no renal tumors were reported. This evidence is suggestive of an inter-species difference in the susceptibility of lead-induced renal tumors.

7.4.2 Candidate Critical Epidemiological Studies

Decision-making criteria (described above) were applied to assess the quality of individual studies for lung, stomach and all-site cancers in order to select candidate studies from which to derive a quantitative estimate of the carcinogenic potency of lead. Two candidate critical epidemiological studies emerged following application of decision-making criteria; one for all-site cancer (Schober *et al.* (2006)) and the other for lung cancer (Englyst *et al.* (2001)). These studies were selected because the weight-of-evidence highlighted the strongest association between lead exposure and three different cancer endpoints - lung, stomach and all-site cancers. A critical study was not identified for stomach cancer because studies the available studies did not meet the strongest evidence for an association between lead exposure and stomach cancer, but a nested case control study conducted in the same study did not support this association. The study authors noted a higher number of Irish- or Italian-born workers amongst cases as compared to controls, suggesting possible confounding by place of birth, potentially related to *H. pylori* infection status and/or diet.

Schober *et al.* (2006) conducted a retrospective cohort study based on NHANES III (1988-1994) data and considering all-site cancer. Members of the general public were selected for the cohort and the exposure type was environmental. A sub-cohort of 9,686 individuals greater than or equal to 40 years of age was selected for analysis. Passive mortality follow-up was conducted until December, 2000 and 543 deaths were reported. Single blood lead measurements were available for all sub-cohort members, and reported blood lead levels were categorized into the following groupings: $<5 \mu g/dL$, $5 - <10 \mu g/dL$, and $\ge 10 \mu g/dL$. The authors adjusted relative risks for sex, race/ethnicity, education and smoking status. Results support an exposure-response relationship in that they present sequentially increasing, statistically significant (p < 0.01) relative risks (RRs) at three different blood lead groups: RR=1.0 ($<5 \mu g/dL$;

referent level), RR=1.44 (1.12-1.86; blood lead 5 - 9 μ g/dL), and RR=1.69 (1.14-2.52; blood lead \geq 10 μ g/dL).

Similar to Schober *et al.* (2006), Menke *et al.* (2006) analyzed the NHANES III cohort data, considering all-site cancer, and reported no significant increase in cancer mortality for a sub-cohort of individuals with blood lead < 10 µg/dL. Passive mortality follow-up was also conducted until December, 2000. Notably, while Menke *et al.* (2006) considered adults aged 20 years and over, Schober *et al.* (2006) restricted their analyses to adults 40 and over. The mean age of Menke *et al.* (2006) was much younger at 44.4 years, as compared to 57 - 62 years for Schober *et al.* (2006). The Menke *et al.* (2006) study also had a larger sample size with 13,946 participants as compared to 9,757 participants in Schober *et al.* (2006), and, thus, had somewhat more power to detect an effect. However, Schober *et al.* (2006) examined a wider range of exposure (with 617 subjects > 10 µg/dL), and they excluded younger life stages (where higher lead exposures might be less likely to result in cancer mortality due to a reduced opportunity for the observation of latent effects, and the general better health of younger adults. On balance, the Schober *et al.* (2006) study design appears to be more sensitive to cancer mortality than Menke *et al.* (2006).

Englyst *et al.* (2001) conducted a retrospective cohort study of 3,979 primary copper and lead smelter workers in Sweden first employed for a minimum of one year from 1928-1979 and examining a series of cancer endpoints. The exposure type was occupational and two subcohorts were selected for further analysis: subcohort one consisted of 710 workers employed in the lead department and also employed at other smelter work places, while subcohort two consisted of 383 workers employed in the lead department but never at other work places where excess lung cancer risk was previously calculated. Passive cancer follow-up was conducted from 1958-1987. Lead exposure was estimated using a cumulative blood lead index (CBLI), which is an integration of an individual's annual mean blood lead levels. Concurrent exposure to arsenic was noted in the study and arsenic exposure was estimated via company occupational records and spot air sampling to be close to the occupational exposure limits of 500 µg/m³ for 1940-1975, and 50 µg/m³ for 1975-1987. Standardized incidence ratios (SIRs) were not adjusted for covariates. Ten cases of lung cancer were reported in subcohort one, possibly the subcohort less exposed to lead, with a statistically significant SIR of 2.4. Five cases of lung cancer were reported in subcohort two, possibly the subcohort more exposed to lead, with a statistically significant SIR of 3.6. The county population was used as a referent group for calculation of SIRs. The authors noted that concurrent exposure to arsenic likely played a significant role in the lung cancer excess risks, and that it was not possible to separate the carcinogenic effects of lead from that of arsenic. An exposure-response relationship is not distinguishable from the study data, and the occupational exposure is of questionable applicability to the typical lower-dose environmental exposures typical of members of the general population.

The same Swedish cohort used in Englyst *et al.* (2001) was used in a retrospective cohort study in Lundstrom *et al.* (1997) and in a nested case control study in Lundstrom *et al.* (2006). The Lundstrom *et al.* (1997) study was very similar to Englyst *et al.* (2001), but included different subcohorts, and provided less information about the role of concurrent exposure to arsenic as a potential confounder. Statistically significant SIRs of 2.9 and 3.1 were calculated for a subcohort of lead exposed workers, and a subcohort of "lead only workers," respectively. The Lundstrom *et al.* (2006) study matched the lung cancer cases in Lundstrom *et al.* (1997) and Englyst *et al.* (2001) with smelter worker controls based on age, and employed additional measures of exposure to lead for analysis. Calculated odds ratios from this case-control analysis did not support an association between lung cancer and lead exposure. The weight of evidence from this Swedish occupational cohort can be summarized as presenting equivocal evidence for an association between lead exposure and lung cancer.

Schober *et al.* (2006) and Englyst *et al.* (2001) were eliminated from further consideration as candidate critical epidemiological studies from which to derive quantitative estimates of cancer potency.

The strength of the evidence of Schober *et al.* (2006) is partially limited by two considerations: 1) lead exposure was quantified by a single blood lead measurement and this raises the issue of the accuracy of the exposure estimate –the possibility that the observed biomarker-response relationship was not caused by relatively higher blood lead concentrations experienced earlier in life therefore cannot be ruled out. 2) The positive results of Schober *et al.* (2006) are in conflict with the null results reported by Menke *et al.* (2006). These limitations were weighed against the time and resource requirements to develop a covariate adjusted dose-response model suitable for deriving quantitative estimate of cancer potency. It was decided that, given these specific limitations of the Schober *et al.* (2006) data and the larger context of relatively weak evidence of an epidemiological association between lead exposure and cancer, it would not be time or cost effective to develop a covariate adjusted dose-response model suitable for deriving quantitative estimate of cancer potency on the basis of the Schober *et al.* (2006) data.

Englyst *et al.* (2001) was also eliminated from further consideration due to: 1) possible confounding by co-exposure to arsenic; 2) the lack of an observed exposure-response relationship; 3) the negative association between lung cancer and lead exposure observed in the later case control study; and, 4) the questionable applicability of higher dose occupational exposure evidence to the lower-dose environmental exposures typical of members of the general population.

In summary, while the weight of the epidemiological evidence was sufficient to show a limited association between lead exposure and cancer, it was not possible to identify a

critical epidemiological study sufficiently suitable for deriving a quantitative estimate of cancer potency. The criteria used to screen and ultimately reject the epidemiological literature included strength of association, presence/absence of a dose-response relationship, statistical significance, reliability of exposure data, the representative nature of results and control of possible confounding.

7.5 QUANTITATIVE ESTIMATES OF CANCER POTENCY

Health Canada quantifies the potency of a carcinogen by deriving a Tumorigenic Dose $05 (TD_{05})$ or Tumorigenic Concentration $05 (TC_{05})$. A TD_{05} or TC_{05} is the exposure dose or concentration that induces a 5% increase in the incidence of, or deaths due to, tumours considered to be associated with exposure, observed in epidemiological studies in human populations or bioassays in experimental animals is the total intake or concentration associated with a 5% increase in excess risk of exposure related precancerous lesions or tumours. The general procedure for calculating a TD_{05} or TC_{05} is:

- Selection of a critical study;
- If the critical study is an animal cancer assay, scale the doses as appropriate to human equivalent doses;
- Develop a biologically plausible continuous dose-response model for the data of the critical study; and,
- Use the modeled dose-response relationship to calculate the exposure dose or concentration that is associated with a 5% increase in tumors.

The TD₀₅ or TC₀₅ provides a quantitative estimate of cancer potency that is within or close to the experimental range. This approach is favoured by Health Canada for several reasons, including the ability to quantify cancer risk without introducing the uncertainties associated with low-dose extrapolation procedures. The TD₀₅ or TC₀₅ is

not based on the lower confidence limit of the dose or concentration associated with a 5% increase in response, but is computed directly from the dose-response curve. This approach is considered appropriate because standard methods for the calculation of a lower confidence limit ignore the uncertainty in the measurement of the independent variable (dose or exposure concentration) and therefore the precision of the confidence interval (i.e. 95% lower confidence interval) is over stated. As the uncertainty in the measurement of the independent variable increases, the confidence limits around a dose associated with a specified response will increase (Greene and Ernhart, 1993; Budtz-Jorgensen *et al.*, 2004).

Waalkes et al. (1995)

 $TD_{01}s$, $TD_{05}s$, and unit risks were calculated for the Waalkes *et al.* (1995) mouse bioassay data. Experimental concentrations in ppm were converted to units of mg/kg/day by assuming a mouse weighs 25g and drinks 5 ml water/day, resulting in experimental doses of 100, 150 and 200 mg/kg/day. The linearized multistage (LMS) method was used in this report to calculate unit risks and a multistage model was used to calculate $TD_{01}s$ and $TD_{05}s$. Mouse to human kinetic adjustment factors were applied to the final potency values. Final unit risks are presented with and without this adjustment factor.

The multistage model is given by Equation 10.

Equation 10

$$P(d) = 1 - e^{-q_0 - q_1 d - \dots - q_k d^k}$$

where *d* is dose, *k* is the number of dose groups in the study (excluding control), P(d) is the probability of the animal developing an endpoint at dose *d* and $q_i > 0$, *i*=0,...,*k* are parameters to be estimated. The unit risk is defined as the increase in excess risk per unit dose, where excess risk is given by Equation 11.

Equation 11

$$\frac{P(d) - P(0)}{1 - P(0)}$$

The unit risk is applicable at very low doses, presumably in the range where humans will be exposed. For a small dose, *d*, the excess risk can be shown to be approximately equal to q_1d . Thus, when the background P(0) is small, q_1 represents the slope (i.e. change in risk per increase of unit dose) of the dose-response curve in the low-dose region. In practice, the upper 95% confidence limit on q_1 is used and is denoted by q_1^* . This is the unit risk for the LMS method.

The multistage model can also be used to compute a TD₀₅ by solving Equation 12.

Equation 12

$$\frac{P(TD_{05}) - P(0)}{1 - P(0)} = 0.05$$

for TD₀₅. The tumorigenic dose low 05 (TDL₀₅) is the 95% statistical lower confidence bound on the TD₀₅. For a cancer endpoint, both the TD₀₅ and the TDL₀₅ can be used to estimate the unit risk (q^*) by 0.01/TD₀₁ or 0.05/TDL₀₅. Similar calculations can be performed for TD₀₁s and TDL₀₁s. The multistage models were fit using THRESH (Howe, 1995) and the unit risks and TDs were calculated. A chi-square lack of fit test was performed for each of the model fits. The degrees of freedom for this test are equal to k minus the number of q_i 's whose estimates are non-zero. A p-value less than 0.05 indicates a significant lack of fit.

Results from the model fitting, along with the applicable potency estimates, are displayed in Table 28 and Table 29. None of the models exhibited significant lack of fit. The multistage model fit and the TD_{01} and TD_{05} for renal tubular cell adenomas in male mice are illustrated in Figure 36 and Figure 37, respectively.

Mouse to human kinetic conversions were applied to the lifetime unit risks by dividing the unit risks by Equation 13.

Equation 13

$$BSA = \left(\frac{0.025kg}{70kg}\right)^{1/4}$$

where 70kg is the body weight of a human, and 0.025 kg is the body weight of a mouse. These are the 'adjusted' unit risks in Table 29.

Table 28. Unadjusted^{*} quantitative estimates of cancer potency of lead derived from a maternal mouse exposure study by Waalkes et al. (2005). The linearized multistage (LMS) method was used to calculate unit risks and a multistage model was used to calculate TD₀₁s and TD₀₅s.

		TD (mg/kg/day)		Unit Risk (mg/kg/d) ⁻¹				
Study/Endpoint	Model	TD ₀₅	TD ₀₁	LMS	0.05/	0.05/	0.01/	0.01/
	P-	(TDL ₀₅)	(TDL ₀₁)		TD ₀₅	TDL ₀₅	TD ₀₁	TDL ₀₁
	value							
Male mice								
Renal tubular cell adenoma	0.22	143.6 (104.8)	83.4 (25.8)	3.85x10 ⁻⁴	3.48x10 ⁻⁴	4.77x10 ⁻⁴	1.20x10 ⁻⁴	3.88x10 ⁻⁴
Renal tubular cell atypical hyperplasia	0.94	72.8 (25.7)	23.8 (5.0)	2.00x10 ⁻³	6.87x10 ⁻⁴	1.95x10 ⁻³	4.20x10 ⁻⁴	1.99x10 ⁻³
RPLs	0.63	66.6 (19.7)	18.9 (3.9)	2.60x10 ⁻³	7.51x10 ⁻⁴	2.54x10 ⁻³	5.28x10 ⁻⁴	2.59x10 ⁻³
Female mice								
RPLs	0.68	143.9 (90.4)	83.6 (18.5)	5.42x10 ⁻⁴	3.48x10 ⁻⁴	5.53x10 ⁻⁴	1.20x10 ⁻⁴	5.40x10 ⁻⁴

Not adjusted for animal to human kinetic differences

Table 29. Adjusted* quantitative estimates of cancer potency of lead derived from a maternal mouse exposure study by Waalkes et al. (2005). The linearized multistage (LMS) method was used to calculate unit risks and a multistage model was used to calculate $TD_{01}s$.

		TD (mg/kg/day)		Unit Risk (mg/kg/d) ⁻¹				
Study/Endpoint	Model	TD ₀₅	TD ₀₁	LMS	0.05/	0.05/	0.01/	0.01/
	P-value	(TDL ₀₅)	(TDL ₀₁)		TD ₀₅	TDL ₀₅	TD ₀₁	TDL ₀₁
Male mice								
Renal tubular cell adenoma	0.22	19.7 (14.4)	11.5 (3.5)	2.80x10 ⁻³	2.53x10 ⁻³	3.47x10 ⁻³	4.36x10 ⁻³	1.41x10 ⁻²
Renal tubular cell atypical hyperplasia	0.94	10.0 (3.5)	3.3 (0.69)	1.45x10 ⁻²	5.00x10 ⁻³	1.42x10 ⁻²	1.53x10 ⁻²	7.22x10 ⁻²
RPLs	0.63	9.2 (2.7)	2.6 (0.53)	1.89x10 ⁻²	5.46x10 ⁻³	1.84x10 ⁻²	1.92x10 ⁻²	9.42x10 ⁻²
Female mice								
RPLs	0.68	19.8 (12.4)	11.5 (2.5)	3.94x10 ⁻³	2.53x10 ⁻³	4.02x10 ⁻³	4.35x10 ⁻³	1.96x10 ⁻²

^{*}Adjusted for animal to human kinetic differences



Figure 36. TD₀₅ and TDL₀₅ for excess risk of renal tubular adenomas in male mice that were exposed to lead in utero and during lactation. The materbal lead doses are in mg/kg/d. The data were modeled using the multistage model and are from Waalkes et al. (1995).



Figure 37. TD₀₁ and TDL₀₁ for excess risk of renal tubular adenomas in male mice that were exposed to lead in utero and during lactation. The maternal lead doses are in mg/kg/d. The data were modeled using the multistage model and are from Waalkes et al. (1995).

Waalkes et al. (2004)

Quantitative estimates of cancer potency were also derived from the lifetime mouse study by Waalkes *et al.* (2004) using the same methods described above. The unadjusted TD_{05} for the most sensitive endpoint (renal proliferative lesions; RPLs) was about three-fold higher (results not shown) than the unadjusted RPL TD_{05} calculated above from Waalkes *et al.* (2005) for male mice with *in utero* and lactational exposures only. These results indicate that the mouse is more sensitive to early life lead exposures. With no human data to confirm or refute this, the same may also be assumed true of humans.

Quantitative Estimates of the Cancer Potency of Lead

A multistage model and a linearized multistage model (LMS) were used to quantify the cancer potency of lead from two murine assays (Waalkes *et al.*, 1995; Waalkes *et al.*, 2004)?. .One of the assays was for perinatal exposure only while the other was from adult exposure only. The quantitative estimates of cancer potency by all methods were higher for male mice from the perinatal exposure assay. The results, adjusted for mice-to-human differences in kinetics, of the quantitative estimates of cancer potency based on the male mouse perinatal exposure are presented in Table 29. The incidence of RPLs, which are understood to be pre-cancerous lesions, was the most sensitive endpoint measured. The adjusted TD₀₅ for RPLs in perinatally exposed male mice calculated from the multistage model is 9.2 mg/kg/d, and the associated cancer unit risk (i.e. oral slope factor) is 5.46 x 10⁻³ (mg/kg/d)⁻¹. The TD₀₁ for the same exposure and endpoint is 2.6 mg/kg/d, and the associated cancer unit risk is 1.92×10^{-2} (mg/kg/d)⁻¹. The LMS for the same exposure and endpoint produced a cancer unit risk of 1.89×10^{-2} (mg/kg/d)⁻¹. In summary, the cancer unit risks for the most sensitive sex and lifestage range from about 5×10^{-3} (mg/kg/d)⁻¹ to 2×10^{-2} (mg/kg/d)⁻¹.

7.6 VARIATION IN SUSCEPTIBILITY TO THE CANCER POTENCY OF LEAD

As mentioned above, there are sex and age specific differences in rodent susceptibility to renal tumors. Males are more sensitive than females and young appear to be more sensitive than adults (Waalkes *et al.*, 1995; Waalkes *et al.*, 2004). Age and sex related sensitivities may be related to age and sex dependent differences in the metal-binding protein metallothionein.

Metallothioneins are low molecular weight cysteine-rich proteins which bind metals. There is evidence that metallothioneins offer a protective mechanism against renal tumors and nephrotoxicity associated with lead exposure (Waalkes *et al.*, 1995; Qu *et al.*, 2002; Waalkes *et al.*, 2004). There are age-dependent changes in metallothionein levels: Yoshida *et al.* (1998) reported lower levels of metallothionein in liver and kidney tissue of young Japanese than adults. In addition, at least two different polymorphisms of the metallothionein-III gene (MT-IIA) have been identified in humans (Yoshida *et al.*, 1998). Up to a 35-fold difference in metallothionein expression in human blood lymphocytes and renal tissues has been reported (Yoshida *et al.*, 1998; Wu *et al.*, 2000). Variation in metallothionein expression among humans may also result in varied susceptibility to the toxicity of lead for these and other endpoints.

Waalkes *et al.* (2004) and Qu *et al.* (2002) demonstrated that metallothionein –I/II knockout mice are much more sensitive than wild-type mice to the carcinogenic and chronic and sub-chronic nephrotoxic effects of lead. The incidence of total renal proliferative lesions in metallothionein-null mice was 3-fold higher than in wild-type mice. There was a reported difference in the severity of lesions too – about 80% of the renal proliferative lesions in wild-type mice were mild hyperplasia whereas 83% of the total renal proliferative lesions in metallothionein-null mice were moderate hyperplasia or tumors. The incidence and severity of lead-induced chronic nephropathy (tubular degeneration, necrosis, mineralization and interstitial fibrosis) was also increased in metallothionein-null mice, whereas no effect was observed in wild-type mice. The knockout mice also had a reduced numbers of nuclear inclusion bodies. This study provides strong evidence that metallothionein inclusion bodies offer a protective mechanism against the nephrotoxicity and renal carcinogenicity of lead.

Summary

No suitable epidemiological study was identified for derivation of quantitative estimates of the cancer potency of lead. Two mouse studies were used to derive quantitative

estimates of the cancer potency of lead. The most sensitive endpoint, life-stage, and sex was excess risk of renal proliferative lesions in male mice that were exposed *in utero* and during lactation only. A multistage model was used to model excess risk of renal proliferative lesions in these animals. The modeled exposure dose, adjusted for human kinetics, associated with a 5% excess risk (TD_{05}) of developing renal proliferative lesions was 9.2 mg/kg/d. The cancer unit risk (slope factor) associated with this TD_{05} is 5.46 x10⁻² (mg/kg/d)⁻¹. Based on this cancer unit risk, Health Canada's existing pTDI for lead of 0.0036 mg/kg/d is associated with an estimated Incremental Lifetime Cancer Risk (ILCR) of approximately 2 in 100,000.

7.7 RELATIVE SENSITIVITY OF THE CANCER ENDPOINT

This section of the report presents an analysis of the relative sensitivity of cancer and the other critical endpoints for lead (IQ decrements and increased SBP). Both qualitative and quantitative comparisons are made. Qualitatively, the relative weight of evidence supporting the presence of low-dose effects for each endpoint was examined. Quantitatively, the most sensitive TD_{01} for carcinogenic effects was compared to benchmark blood lead concentrations (BMCs) derived for the non-cancer effects. The BMCs are equivalent to a 1% increase in the risk of MMR or hypertension or CHD mortality. The O'Flaherty toxicokinetic model for lead was used to estimate the maternal blood lead equivalent to the oral lead dose associated with the TD_{01} for carcinogenic effects.

Quantitative Comparison of Endpoint Sensitivity

A quantitative comparison of endpoint sensitivity is provided by:

- Converting the TD₀₁ (i.e. the dose, adjusted for mouse-to-human kinetics, associated with a 1 in 100 excess risk of renal proliferative lesions (RPLs)) to an equivalent human maternal blood lead concentration.
- Calculating the benchmark responses (BMRs), in terms of absolute change in mean IQ and mean SBP, required to produce a 1 in 100 increase in the risk of mild mental retardation (MMR), hypertension, and coronary heart disease (CHD) mortality.
- Calculating the benchmark blood lead concentrations (BMCs) associated with the BMRs (associated with a 1 in 100 increase in the risk of MMR, hypertension, and CHD mortality).

Human Maternal Blood Lead Concentration Equivalent to the TD₀₁

The lowest (most sensitive) adjusted TD_{01} from the modeling of data from the mouse studies was 2.6 mg/kg/d.

The O'Flaherty model (O'Flaherty, 1998) was used to calculate the blood lead and cortical bone lead concentrations associated with a chronic intake of 2.6 mg Pb/kg/d in drinking water. The default physiological parameters for a woman were used and the model was run for a 17 to 45 year-old female. Lead in drinking water was the only lead source modeled. We assumed a water ingestion rate of 1.5 L/day and, therefore, set the lead concentration in water to a constant value of 104 mg/L to simulate an intake of 2.6 mg Pb/kg/d in drinking water. Modeled blood lead concentrations increased slowly over the simulation period of 17-45 years of age from about 200 μ g/dL to about 250 μ g/dL. Modeled cortical bone lead concentrations increased more rapidly over the simulation period of 17-45 years of age from about 1,000 μ g/g to about 2,500 μ g/g. The

maternal blood lead concentration, therefore, associated with a 1 in 100 excess risk of RPLs in male offspring is estimated to be 200 to 250 µg/dL.

Benchmark Responses Required for a 1 in 100 Risk of Dichotomous Outcomes

The methods described in Appendix Awere used to calculate absolute change in mean IQ and mean SBP required to produce a 1 in 100 increase in the risk of MMR, hypertension, and coronary heart disease (CHD) mortality. The models were originally constructed to calculate the added risk of hypertension and CHD mortality. The added risk of these outcomes will be greater than the excess risk for a given change in mean SBP. The quantitative difference between these definitions of increased risk, however, was assumed to be negligible in the context of the overall uncertainty of the estimates.

- A decrement in population mean IQ of 2.325 IQ points is associated with a 1 in 100 excess risk of MMR.
- An increase in population mean SBP of 0.26 mmHg is associated with a 1 in 100 added risk of hypertension (pre, stage I, and stage II hypertension).
- An increase in population mean SBP of 29 mmHg is associated with a 1 in 100 added risk of CHD mortality.

Benchmark Blood Lead Concentrations Associated with a 1 in 100 Risk of Dichotomous Outcomes

The methods described above were used to calculate the benchmark blood lead concentrations (BMCs) associated with the above BMRs (associated with a 1 in 100 increase in the risk of MMR, hypertension, and CHD mortality). The calculated BMCs are as follows:

- The BMC associated with a decrement in population mean IQ of 2.325 IQ (associated with a 1 in 100 excess risk of MMR) is 0.5 to 3.5 µg/dL.
- The BMC associated with an increase in population mean SBP of 0.26 mmHg (associated with a 1 in 100 added risk of hypertension) is 0.3 to 5.2 µg/dL.
- The BMC associated with an increase in population mean SBP of 29 mmHg (associated with a 1 in 100 added risk of CHD mortality) is 36 to 580 µg/dL, with a best estimate for susceptible sub-populations of 62 µg/dL. This latter BMC is the most appropriate value to compare to the maternal blood lead concentration associated with the TD₀₁ for RPLs in perinatally exposed male offspring (also a susceptible sub-population).

The results of this quantitative comparison are presented in Table 30. The blood lead concentrations associated with a 1 in 100 excess risk of MMR and a 1 in 100 added risk of hypertension are about 50 to 1,000-fold lower than those associated with a 1 in 100 excess risk of RPLs and a 1 in 100 added risk of CHD mortality. The best estimate of the blood lead concentration associated with a 1 in 100 added risk of CHD mortality and the most closely analogous comparator is about three to four fold lower than the blood lead concentration associated with a 1 in 100 excess risk of RPLs.

Endpoint	Associated dose mg/kg/d	Associated blood lead (µg/dL)	Measured response	Modeled response
RPL	2.6	200-250	TD ₀₁ : 0.01 excess risk of RPL	-
MMR	-	0.5 to 3	BMR of 2.325 IQ points	0.01 excess risk of MMR
Hypertension	-	0.3 to 5.2	BMR of 0.26 mmHg	0.01 added risk of hypertension
CHD mortality	-	36 to 580 (best estimate: 62)	BMR of 29 mmHg	0.01 added risk of CHD mortality

Table 30. Blood lead concentrations associated with 1 in 100 risk of cancer and non-cancer outcomes

The results of this quantitative comparison suggest that a 1 in 100 risk of MMR, hypertension, and CHD mortality are associated with lower blood lead concentrations than a 1 in 100 risk of RPLs. The relative importance of these outcomes is a value judgement that should be left to risk managers and stakeholders. This information is presented so that risk assessors can illustrate that the estimated blood lead concentrations required to produce a quantitatively equivalent increase in risk among these endpoints differ by the magnitudes indicated.

- The estimated blood lead concentration associated with a 1 in 100 added risk of CHD mortality amongst susceptible subpopulations is 3 to 4 fold lower than the estimated blood lead concentration associated with a 1 in 100 excess risk of pre-cancerous renal lesions in a susceptible subpopulation.
- The estimated blood lead concentration associated with a 1 in 100 added risk of hypertension is 50 to 1,000 fold lower than the estimated blood lead concentration associated with a 1 in 100 excess risk of pre-cancerous renal lesions in a susceptible subpopulation.
- The estimated blood lead concentration associated with a 1 in 100 excess risk of MMR is 70 to 500 fold lower than the estimated blood lead concentration

associated with a 1 in 100 excess risk of pre-cancerous renal lesions in a susceptible subpopulation.

In addition to these quantitative comparisons among endpoints, risk assessors should also understand and communicate the qualitative differences in the weight of evidence supporting the various quantitative estimates.

Qualitative Comparison of Endpoint Sensitivity

The weight of evidence supporting adverse neurological and cardiovascular effects at blood lead concentrations < 10 μ g/dL is much stronger than the existing evidence supporting an association between cancer and blood lead concentrations < 10 μ g/dL. There are multiple animal studies showing adverse neurological and cardiovascular effects at blood lead concentrations less than or just slightly greater than 10 μ g/dL. There are no animal studies demonstrating carcinogenic effects at doses that would be expected to produce blood lead concentrations less than approximately 50 µg/dL. Additionally, there is strong epidemiological evidence of an association between blood lead concentrations < 10 µg/dL and adverse neurological effects and suggestive epidemiological evidence of an association between blood lead concentrations < 10 µg/dL and adverse cardiovascular effects. The epidemiological evidence of an association between lead exposure and cancer outcomes is relatively weak in comparison. There are plausible and relevant biological mechanisms for all three endpoints, but there is not strong evidence supporting lead as a mutagenic carcinogen at environmentally relevant exposure levels. Therefore, extrapolation of effects below the experimental dose-effects range is uncertain. The unadjusted TD₀₁ is about 5-fold lower than the lowest experimental dose associated with adverse effects, whereas the BMCs for the non-cancer effects are within or very close to within the range of data from the critical studies. Therefore, theweight of evidence supporting the possibility of

the carcinogenic effects of exposure to lead at environmentally relevant levels is much weaker than that for the developmental neurotoxicity and cardiovascular toxicity of lead.

Summary

The modeled blood lead concentrations associated with the most sensitive carcinogenic TD_{01} are three to one thousand-fold greater than the benchmark blood lead concentrations for an equivalent risk of dichotomous non-cancer effects. The weight-of-evidence supporting the possibility of the carcinogenic effects of lead at environmentally relevant exposure doses is also much weaker than that for the developmental neurotoxicity and cardiovascular toxicity of lead.

7.8 CONCLUSIONS

There are multiple lines of evidence, including human epidemiological studies, *in vivo* animal assays, and *in vitro* experiments, that support the hypothesis that lead exposure can induce cancer in humans. The epidemiological evidence of an association between lead exposure and cancer in humans is limited but that there is sufficient evidence to conclude that lead is carcinogenic in animal experiments. Plausible and relevant Modes of Action have been identified. The genotoxicity of lead remains uncertain and can, therefore, not be ruled out.

This section of the report:

Briefly summarized the available evidence from animal cancer bioassays and epidemiological studies of the carcinogenicity of lead and summarize the available evidence of the genotoxic properties of lead from multiple lines of *inquiry*. Lead is a confirmed animal carcinogen but the epidemiological evidence of an association between lead exposure and cancer in humans is limited. There is evidence that lead is genotoxic but insufficient evidence to establish whether lead is directly genotoxic or indirectly genotoxic. The results of assays to test the mutagenicity of lead are inconsistent.

Reviewed the categorization of lead as a human carcinogen, including evaluations by other health agencies, such as the International Agency for Research on Cancer (IARC) and the US National Toxicology Program (NTP) as well as apply Health Canada's criteria for categorization of carcinogens under the Canadian Environmental Protection Act (CEPA). Lead is "likely", "probably" or "reasonably anticipated" to be a human carcinogen. These categories are equivalent to a Group II carcinogen under Health Canada's CEPA categorization criteria. It is Health Canada's policy to quantify the potency of CEPA Group I and II carcinogens and to assess and manage exposure to these substances based on their most sensitive health endpoint.

Reviewed the available information on plausible Modes of Action (MoA) of lead induced carcinogenesis and discuss the implications for defining lead as a threshold or non-threshold carcinogen. The MoA of carcinogenesis of lead is uncertain and there is insufficient evidence to identify a threshold for the carcinogenic effects of lead. Therefore, the following default assumptions apply: the MoA that is operative in animals is also relevant to humans and the cancer risks associated with lead exposure are characterized assuming that the dose-response curve is linear at low doses (i.e. no population threshold for effects).

Identified the critical studies for quantification of the cancer potency of lead. Two studies of renal tumors in mice, one from perinatal exposure (Waalkes *et al.*, 1995) and one from adult lifetime exposure (Waalkes *et al.*, 2004), were identified as candidate

critical animal studies. No epidemiological study suitable for developing a quantitative estimate of the cancer potency of lead was identified.

Described the methods and results of dose-response modeling of the candidate critical studies and the derivation of quantitative estimates of cancer potency from each. The carcinogenic potency of lead was estimated by using a multistage model to derive a Tumorigenic Dose 05 (TD_{05}). A TD_{05} is the dose associated with a 5% increase in excess risk of exposure related pre-cancerous lesions or tumours. The most sensitive life-stage, sex, and endpoint was the excess risk of renal proliferative lesions among male mice exposed *in utero* and lactationaly only. The TD_{05} for this endpoint, adjusted for potential differences in mouse to human kinetics, is 9.2 mg/kg/d. The cancer unit risk (oral slope factor) associated with this TD_{05} is 5.46 x10⁻² (mg/kg/d)⁻¹. Based on this cancer unit risk, Health Canada's existing pTDI for lead of 0.0036 mg/kg/d is associated with an estimated Incremental Lifetime Cancer Risk (ILCR) of approximately 2 in 100,000.

Provided a quantitative and qualitative comparison of the relative potencies of the carcinogenic, cardiovascular, and neurotoxic effects of lead. The estimated blood lead concentration associated with a 1 in 100 added risk of CHD mortality amongst susceptible subpopulations is 3 to 4 fold lower than the estimated blood lead concentration associated with a 1 in 100 excess risk of pre-cancerous renal lesions in a susceptible subpopulation.

 The estimated blood lead concentration associated with a 1 in 100 added risk of hypertension is 50 to 1,000 fold lower than the estimated blood lead concentration associated with a 1 in 100 excess risk of pre-cancerous renal lesions in a susceptible subpopulation.

- The estimated blood lead concentration associated with a 1 in 100 excess risk of MMR is 70 to 500 fold lower than the estimated blood lead concentration associated with a 1 in 100 excess risk of pre-cancerous renal lesions in a susceptible subpopulation.
- The weight of evidence supporting the carcinogenic effects of lead at environmentally relevant exposures is weaker than that for the developmental neurotoxicity and cardiovascular toxicity of lead.

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APPENDIX A

Population Health Risks Associated with a 1% Benchmark Response

Note: the tables for Appendix A appear at the end of the section.

A relative Benchmark Response (BMR) of 0.01 (e.g. 1 % percent change in mean response relative to an unexposed population) was selected as the continuous Benchmark Response (BMRc) for deriving benchmark blood and bone lead reference concentrations. This appendix presents the methods and results of modeling that expressed a 1% decrement in population mean IQ and a 1% increase in population mean systolic blood pressure (SBP) in terms of their expected population health effects. The primary purpose of this modeling is to demonstrate that a 1% change in either of these endpoints is toxicologically relevant from a population health perspective and make the case that a 0.01 relative BMR is an appropriate and justifiable BMR for these endpoints. These models can also be used to express changes in population mean IQ or SBP as associated changes in population morbidity and mortality. These endpoints can further be monetized or otherwise used to support policy decisions around tolerable levels of environmental lead exposure for the Canadian population.

Appendix A will:

Introduce normal distribution theory. Normal distribution theory is a method of expressing a continuous endpoint, such as IQ or blood pressure, as a probability of exceeding a predefined cut-off on the continuous distribution that defines a quantal adverse outcome. e.g. a systolic blood pressure > 140 mmHg is defined as hypertension.

Describe the methods and results of the application of the normal distribution theory to calculate the increased risk of Mild Mental Retardation (MMR) among
children associated with a decrement in population mean IQ of 1%. The results of this modeling exercise are that a 1% decrement in population mean IQ is associated with an added population risk of MMR of approximately 1 in 250 (385 in 100,000) children.

Describe the methods and results of application of the normal distribution theory to calculate the increased probability of hypertension among adults 35-74 years old associated with an increase in population mean systolic blood pressure (SBP) of 1%. The total sex and age-adjusted added risk of hypertension (Pre, Stage I and Stage II) among 35-74 year-olds associated with a 1% increase in population mean SBP is approximately 1 in 20 (5,421 per 100,000).

Describe the methods and results of a Relative Risk model to calculate the incremental cumulative risk of Coronary Heart Disease (CHD) mortality for adults 35-74 years old associated with an increase in population mean SBP of 1%. The sex and age-adjusted cumulative (35-74 years) incremental risk of CHD mortality associated with a 1% increase in population mean SBP is 1 in 2,000 (50 per 100,000). Males are much more sensitive that females and constitute about 80% of the calculated CHD deaths

Reach a conclusion, from a population health perspective, on the toxicological significance on a 1% decrement in population mean IQ and a 1% increase in population mean systolic blood pressure. The conclusions are that a 1% change in both of these continuous endpoints is toxicologically significant on a population level and an appropriate Benchmark Response upon which to base a toxicological reference value for the prevention of unacceptable adverse population health effects.

7.9 ACKNOWLEDGEMENTS

The relationship between a lead related change in population IQ and the prevalence of children with MMR has previously been discussed and modeled by a number of authors, including (Weiss 2000; Fewtrell et al. 2004). Fewtrell et al. (2004) also modeled cardiovascular morbidity associated with lead exposure.

Modeling of lead-related quantal health effects was originally completed for Health Canada by Equilibrium Environmental Inc. Equilibrium's report (Equilibrium Environmental Inc. 2008a) was peer reviewed and is available upon request to Health Canada, Safe Environments Programme, Bureau of Risk and Impact Assessment, Ottawa, ON, Canada or by emailing cs-sc@hc-sc.gc.ca. The mathematical models described in this appendix were developed in Microsoft Excel by Health Canada and are also available upon request. Some of the text in the sections below on systolic blood pressure and hypertension and hypertension related coronary heart disease mortality have been paraphrased from Equilibrium Environmental Inc. (2008a). The preliminary model development work by Equilibrium Environmental Inc. (2008a) peer review panel and the Health Canada Lead (Pb) Issues Working Group are gratefully acknowledged.

7.10 NORMAL DISTRIBUTION THEORY & QUANTAL POPULATION HEALTH ENDPOINTS

Normal distribution theory may be used to define the population health effects associated with either an increase in the population mean systolic blood pressure of 1% or a decrement in the population mean IQ of 1%. The normal distribution theory is used to determine the change in the probability of a quantal adverse outcome, such as mild mental retardation or stage I systolic hypertension, based on a change in the population mean of a continuous toxicological endpoint, such as IQ or systolic

blood pressure. Because benchmark blood and bone lead reference concentrations will be used for primary prevention (i.e. for determining maximum allowable population lead exposures from all environmental media for which Health Canada regulates or establishes national guidelines), the definition of what constitutes a toxicologically relevant response was approached from a population health perspective. In other words, the intent of this line of inquiry was to estimate some of the expected national population health outcomes associated with a 1% change in population mean IQ and systolic blood pressure. The population health endpoint that was calculated in association with a decrement in IQ was the prevalence of mild mental retardation (defined as a IQ < 70). The population health endpoints that were calculated in association with an increase in systolic blood pressure (SBP) were the prevalence of stage 1 systolic hypertension (defined as SBP > 140 mmHg) and coronary heart disease (CHD) mortality.

Change in probability of a quantal adverse effect

A cut-off value that distinguishes a normal response from an adverse response must be identified in order to use data from a continuous endpoint to model the probability of a quantal adverse response. This can be achieved by defining x_0 , the absolute cut-off in response that defines normal or adverse responses, or indirectly by specifying the probability of an adverse responses expected among unexposed or baseline subjects, P(0).

For continuous endpoints, such as IQ, blood pressure, organ weight, body weight, etc., there generally is no sharp demarcation between normal and adverse values. In the absence of a clinical definition of an adverse value, a low or high percentile (e.g. the 1st or 99th percentile) of the population distribution of the response could be defined as adverse. For continuous variables that are normally distributed, these percentiles are equal to the mean ± 2.33 standard deviations. For IQ, the clinical

definition of MMR (IQ< 70) was used to define an adverse response. For SBP, the clinical definition of Stage I Systolic Hypertension (SBP > 140 mmHg) was used to define an adverse response. For modeling CHD mortality risk, the additional clinical definitions of pre-hypertensive (SBP > 130 mmHg and Stage II Systolic Hypertension (SBP > 160 mmHg) were also used.

For normally distributed response data with a constant variance, the probability of a quantal adverse response can be expressed as described in Equation A1, when the response decreases with increasing dose, or Equation A2, when the response increases with increasing dose.

Equation A1

$$P(BMR_c) = \theta \left[\frac{x_0 - (\mu(0) - (\mu(0) \times BMR_c))}{\sigma} \right]$$

Equation A2

$$P(BMR_c) = 1 - \theta \left[\frac{x_0 - (\mu(0) + (\mu(0) \times BMR_c))}{\sigma} \right]$$

Where

 θ = the standard normal distribution function;

- x₀ = the cut-off on the normally distributed endpoint that defines a case or adverse response
- $\mu(0)$ = the mean response of the population at 0 exposure

- BMRc = the relative Benchmark Response for the continuous response (i.e. % change in continuous response)
- σ = the standard deviation of the continuous response

IQ and Mild Mental Retardation

Mild Mental Retardation (MMR) is defined as an IQ < 70. To assist in demonstrating the toxicological significance of a mean decrement in population IQ of 1% (equivalent to 1 IQ point) from a population health perspective, the normal distribution theory was used to calculate the associated added risk and extra risk of MMR as well as the increase in prevalence of MMR.

Added risk and extra risk of MMR were calculated according to Equations A3 and A4, respectfully. The mean IQ of the comparator population with no lead exposure was assumed to be 100 IQ points and the standard deviation of both the comparator population and the lead exposed population was assumed to be 15 IQ points.

Equation A3

additional risk = $P(BMR_c) - P(0)$,

Equation A4

 $\operatorname{extra} \operatorname{risk} = \frac{P(BMR)_c - P(0)}{1 - P(0)},$

Where

P(0) = the probability of an adverse response (MMR) at 0 exposure

Additional risk is an absolute increase in risk whereas extra risk is an increase in risk relative to the probability of being unaffected in the unexposed group. Extra risk can also be thought of as the added risk among those that would have been unaffected had they not been exposed (Bailer et al. 1997). Extra risk is always at least as large as additional risk and the 2 models are equal when background risk P(0) is equal to zero.

Prevalence of MMR per 100,000 people was calculated by multiplying the risk of MMR at a given exposure, (P(0) or P(BMRc), by 100,000.

The results of this modeling are presented in Table A1 and Figure A1 below. For purposes of illustration, the added and extra risk of MMR and the change in prevalence of MMR was calculated for a 0.1%, 1%, 5% and 10% change in population mean IQ. The relative continuous Benchmark Response (BMRc) selected for the derivation of Health Canada's Benchmark blood lead concentration is 0.01, or 1%. A 1% decrement in population mean IQ is associated with an added risk of MMR of approximately four in one thousand. As a point of comparison, the range of de minimus incremental lifetime cancer risks (ILCR) inherent in many Health Canada environmental quality guidelines for the protection of population health is one in ten thousand to one in one million. The added risk of MMR associated with a population mean decrement in IQ of 1% (or 1 IQ point) is approximately 40 to 4,000 times greater than acceptable ILCR that is inherent in many Canadian and international environmental quality guidelines and standards for the protection of population health effects. Therefore a continuous benchmark response (BMRc) of 0.01, or a 1% decrement in population mean IQ, is judged to be toxicologically significant from a population health perspective and is a justifiable BMRc to derive toxicological reference values for the prevention of significant adverse population health effects.



Figure A1. Estimated Increase in prevalence of population MMR (cases per 100,000) as a function of decrements in population mean IQ, assuming that IQ is normally distributed and has a constant variance. A 1% decrement in population mean IQ is associated with an increase in the prevalence of MMR of 385 cases per 100,000.

Systolic Blood Pressure and Stage I Hypertension

A lead-related change in the population mean SBP can also be expressed via the normal distribution theory as added or extra risk of hypertension. This section describes the methods and results of modeling used to determine the sex and age-specific (unadjusted) added risk and extra risk of Stage I Systolic Hypertension (SBP > 140 mmHg) associated with a 1% increase (a relative continuous Benchmark Response of 0.01) in the mean Canadian SBP. In addition to modeling added and

extra risk of Stage I Systolic Hypertension, the sex-specific age adjusted additional prevalence of Stage I Systolic Hypertension was also calculated. These endpoints were calculated via the following 3 steps:

- Use the normal distribution theory to calculate sex and age-specific probabilities of SBP > 140 mmHg among baseline populations and among response populations with a 1% increase in population mean SBP
- Use the probabilities calculated in step 1 to calculate the sex and age-specific added risk and extra risk of Stage I Hypertension associated with a 1% increase in population mean SBP
- Convert the probabilities of Stage I Systolic Hypertension calculated in step 1 to prevalence of Stage I Systolic Hypertension and use Canadian demographic statistics to calculate the sex-specific age corrected prevalence of Stage I Hypertension associated with a 1% increase in population mean SBP

These steps are explained in more detail below.

Baseline Canadian Distributions of SBP

Estimates of Canadian distributions of SBP were used for modeling. Canadian age and sex-specific distributions of SBP were obtained by Equilibrium Environmental Inc. from the Canadian Health Heart Database (Canadian Heart Health Database 1992) and are summarized in Table A2 below. Data were collected over the period of 1986-1992. Data for ages 75 to 84 years were available for Nova Scotia only. These values were used as inputs for the sex and age specific mean SBP of the baseline population ($\mu(0)_i$) and the standard deviation (σ_i) of SBP in both the baseline and response populations. Equilibrium Environmental Inc. (2006) conducted an analysis of the shape of the distribution of SBP data and recommended that a normal distribution was a reasonable approximation.

Gender and Age Specific Probabilities of Stage I Hypertension

Gender and age-specific probabilities of SBP > 140 mmHg or Stage I Systolic Hypertension ($P_I(BMR_c)$) were calculated for the continuous benchmark response of a 1% increase in population SBP via Equation A5.

Equation A5

$$P_{I}(BMR_{c})_{i} = 1 - \theta \left[\frac{x_{0I} - (\mu(0)_{i} + (\mu(0)_{i} \times BMR_{c}))}{\sigma_{i}} \right]$$

Where

i = the ith sex-specific decade age bracket

 θ = the standard normal distribution function

 x_{01} = the cut-off on the distribution of SBP that defines Stage I Systolic Hypertension (140 mmHg)

 $\mu(0)_i$ = the mean SBP of the population at 0 exposure of the ith sex-specific decade age bracket

BMR_c = the relative Benchmark Response for the continuous response (0.01)

 σ_i = the standard deviation of the distribution of SBP of the ith sex-specific decade age bracket

The results of this intermediate calculation are not discussed here, but are presented in Table A3.

Added Risk and Extra Risk of Gender and Age-Specific Stage I Systolic Hypertension

Gender and age-specific estimates of added risk and extra risk of Stage I Systolic Hypertension were calculated by Equation A3 and A4, respectively. The results are presented in Table A3. The age-specific added risk of Stage I Systolic Hypertension for males associated with a 1% increase in age-specific increase in population mean SBP ranged from 1.2E-02 (or 1.2 in 100) to 3.0E-02; the extra risks were slightly higher. The age-specific added risk of Stage I Systolic Hypertension for females associated with a 1% increase in age-specific increase in population mean SBP ranged from 5.6E-04 (or 5.6 in ten thousand) to 3.3E-02; the extra risks were also slightly higher.

Added Prevalence of Gender and Age-Specific Stage I Systolic Hypertension

The sex and age-specific prevalence of Stage I Systolic Hypertension per 100,000 were calculated by multiplying the probability of Stage I Systolic Hypertension at a given response, (P(0) or P(BMR_c), by 100,000. Added prevalence of sex and age-specific Stage I Systolic Hypertension due to a 1% increase in SBP was calculated by finding the difference. These operations were performed by Equations A6 and A7, respectively.

Equation A6

 $C_{I}(0)_{i} = P_{I}(0)_{i} \times 100,000$ or $C_{I}(BMR)_{i} = P_{I}(BMR_{c})_{i} \times 100,000$

Where

 $C_{I}(0)_{i}$ =the prevalence of Stage I Systolic Hypertension for the ith sex-specific decade age bracket at 0 exposure

 $C_{I}(BMR)_{i}$ =the prevalence of Stage I Systolic Hypertension for the ith sex-specific decade age bracket with an increase in population SBP equal to the continuous Benchmark Response (1%)

Equation A7

 $\Delta C_I (BMR)_i = P_I(0)_i - C_I (BMR)_i$

Where

 $\Delta C_{I}(BMR)_{i}$ = the added prevalence of Stage I Systolic Hypertension for the ith sexspecific decade age bracket with an increase in population SBP equal to the continuous Benchmark Response (1%) for the ith sex-specific decade age bracket.

The age and sex-specific added prevalence of Stage I Hypertension were age corrected using the direct method via Equation A8 and Canadian population distribution statistics from 1997 (Statistics Canada 1999).

Equation A8

 $A\Delta C_I(BMR)_i = \Delta C_I(BMR)_i \times A_i$

Where

 A_i = The proportion of the total male or female Canadian population between 35 and 75 years-old for the ith sex-specific decade age bracket

The resulting values represent, for males and females respectively, the added prevalence of Stage I Hypertension per 100,000 males or females 35-75 years-old associated with a 1% increase in age and sex-specific population mean SBP of 1%.

Age-adjusted results were calculated for the age range of 35-75 years only. Estimates of lifetime cancer risk based on Human Health Risk Assessment protocols in Canada typically consider an adult up to the age of 75 years. The exclusion of younger ages (< 35 years) in the calculation of SBP-related Stage I Hypertension prevalence has only a minor impact on the quantitative estimates because these lifestages have a relatively low base-line SBP. While it is acknowledged that the exclusion of older ages (75+ years) underestimates the added prevalence of Stage I Hypertension, this upper cut-off was maintained in order to maintain consistency with the typical cancer risk assessment methods. Additionally, the baseline mean and standard deviation SBP for 75-84 year-olds were from Nova Scotia data only and, therefore, may be less representative of the entire Canadian population than the data for the other age brackets.

The results of this modeling are presented in Table A3 and Figure A2 below. The calculated risks of Stage I Systolic Hypertension are generally greater for males, although this is reversed for the highest age bracket . For males, the most sensitive age bracket is 55-64 year olds, where a 1% increase in SBP is associated with an increase in prevalence of Stage I Systolic Blood Pressure of 3,199 cases per 100,000, or about 1 in 30. For females, the most sensitive age bracket is 75-84 year olds – who have a similar magnitude of increase in prevalence of Stage I Systolic Blood Pressure of Stage I Systolic Blood Pressure age bracket is 75-84 year olds.

A 1% increase in population mean systolic blood pressure is associated with an age corrected sex-specific increase in the prevalence of Stage I Systolic Blood Pressure of 159-751 cases per 100,000 adults (35-74 years old). For the most sensitive age brackets, this represents a population health risk that is 15-1,500 fold higher than the range of ILCR (one in ten thousand to one in one million) inherent in many Health Canada environmental quality guidelines for the protection of population health. Therefore a continuous benchmark response (BMR_c) of 0.01, or a 1% increase in SBP is judges to be toxicologically significant from a population health perspective and is a justifiable BMRc to derive toxicological reference values for the prevention of significant adverse population health effects.



Figure A2. Estimated Increase in prevalence of population Stage I Systolic Blood Pressure (sex-specific cases per 100,000 decade age-bracket) associated with a 1% increase in population mean systolic blood pressure, assuming that systolic blood pressure is normally distributed and has a constant variance. A 1% increase in population mean systolic blood pressure is associated with a sex-specific unadjusted increase in the prevalence of Stage I Systolic Blood Pressure of 56 to 3,272 cases per 100,000 decade age-bracket, depending on age and sex. The advantage of modeling the risk of hypertension associated with changes in SBP, is that it is a relatively simple model. The disadvantage is that hypertension itself is not necessarily an adverse health outcome: The importance of hypertension is its role in the aetiology of more costly and injurious diseases, such as cardiovascular disease, renal dysfunction and dementia.

High blood pressure is a well established and strong risk factor for cardiovascular disease – the leading cause of mortality in North America. Hypertension contributes to all of the major atherosclerotic cardiovascular disease outcomes and can increase the risk of these diseases by 2- to 3-fold. Coronary disease is the most common and the most lethal sequela of hypertension. Hypertension commonly occurs as isolated systolic hypertension. Therefore, the risk of coronary heart disease (CHD) mortality was identified as a suitable population health endpoint to model in association with changes in population mean systolic blood pressure.

A 1% increase in population mean SBP was calculated to determine estimates of increased CHD mortality risk. Modeling CHD mortality risks was conducted via the following steps:

- Use the normal distribution theory to calculate sex and age-specific probabilities of all stages of hypertension among baseline populations and among response populations with a 1% increase in population mean SBP
- Convert the probabilities of various stages of hypertension calculated in step 1 to prevalence of hypertension among the response population
- Use Canadian data on baseline CHD mortality and reported Relative Risks for CHD associated with stages of hypertension to calculate the additional CHD mortality associated with the additional prevalence of hypertension among the response population
- Use Canadian demographic statistics to calculate the sex and age corrected additional CHD mortality for the response population

These steps are explained in more detail below. All of the inputs, default values and outputs for this modeling are presented in Tables A7-A9.

SBP and Prevalence of Pre, Stage I and Stage II Hypertension

The calculation of sex and age-specific probabilities of all stages of hypertension among baseline populations and among response populations associated with a 1% increase in population mean SBP and the conversion of these probabilities into estimates of the prevalence of hypertension was done as was done for the modeling of Stage I Hypertension, except that all stages of hypertension were calculated.

The following SBP cut-off values were used for the respective stages of hypertension:

Pre-Hypertension: x_{0P} = 130 mmHg

Stage I Hypertension: $x_{01} = 140 \text{ mmHg}$

Stage II Hypertension: x_{0II} = 160 mmHg

Thereby, the age and sex-specific probabilities of SBP > 130 mmHg, SBP > 140 mmHg and SBP > 160 mmHg were calculated for the baseline population and for a response population with an increase in age and sex specific SBP of 1%. The probabilities were converted to prevalence by multiplying by 100,000 and the added prevalence of each stage of hypertension was derived by finding the difference between the baseline and response populations.

CHD Mortality Risk as a Function of SBP

The additional cases of Pre, Stage I and Stage II Hypertension are associated with an increased risk of CHD mortality that is incremental to the baseline risk of CHD mortality. Canadian CHD mortality was used as the baseline CHD mortality in the model.

Canadian age and sex-specific cardiovascular mortality data from 1997 are reported in Heart and Stroke Foundation of Canada (HSFC; 1999). These data are presented in Table A5. The reported annual mortalities for ischemic heart disease $(IHD)^{19}$ were multiplied by 10 to derive age and sex-specific baseline CHD mortality per 10 years, M_i(0).

The risk of related sequelae appears to increase continuously with increasing SBP, and researchers have reported increased risk of CHD as both continuous and categorical functions of SBP. For example; studies have described an increase in CHD risk associated with a shift from the normal to pre-hypertensive state (SBP < 130 mmHg to > 130 mmHg) and from Stage I to Stage II hypertension (SBP < 160 mmHg to > 160 mmHg) (Grundy et al. 1999; Domanski et al. 2002; Lloyd-Jones et al. 2005). To simplify the present modeling exercise, CHD risk was calculated as a categorical function of SBP. It is expected that a continuous model would provide comparable estimates of CHD risk.

The risk of CHD morbidity as a function of SBP has been reported in a number of epidemiological studies. The categorical risk functions from the Framingham Heart Study were used in this modeling exercise. The Framingham risk factors were preferentially selected because of the long follow-up period of this study and because other published validation efforts have tested the suitability of these risk

¹⁹ Synonymous with coronary heart disease

factors for diverse populations of varying ethnicity. In general the Framingham risk factors appear to over-estimate CHD risks by up to 50% (Gamblin et al. 1994; Grundy et al. 1999; D'Agostino et al. 2001; Hense et al. 2003) but some of this may be attributed to differing definitions of CHD as most other study cohorts tend to use more narrow definitions of CHD than the Framingham study.

The Framingham Heart Study, centred in Framingham, MA, has followed a cohort of almost exclusively Caucasian males and females for over 50 years with the objective of identifying common (risk) factors or characteristics that contribute to cardiovascular disease. Over 10,000 subjects have been included and the longitudinal study now spans 3 generations.

Ten year CHD risks from the Framingham Heart Study cohort are presented by Grundy et al. (1999) as categorical functions of SBP and other major independent risk factors for CHD. The Framingham definition of CHD includes angina pectoris, recognized and unrecognized myocardial infarction, coronary insufficiency and CHD mortality. The Framingham Relative Risks were based on risk of CHD incidence over 10 years and are relative to the CHD incidence observed in the lowest risk strata in the cohort (for SBP, this would be SBP < 130 mmHg).

While CHD is typically not associated with any one risk factor in isolation, studies have shown that the major independent risk factors can be considered additive in predictive power (Grundy et al. 1999). Therefore the change in Relative Risk (RR) for CHD, as determined by a change in SBP only, was extracted and these values were used in isolation the present modeling exercise. It was further considered reasonable to apply the RR for CHD morbidity to Canadian baseline data because, as noted by (Grundy et al. 1999), although baseline rates of absolute risk of CHD

vary widely, the relative risks of CHD attributable to the major independent risk factors has been found to be relatively universal.

Grundy *et al.* (1999) presents a method for determining the RR for the 10 year incidence of CHD as quantal functions of SBP and other major independent risk factors such as smoking, elevated serum total cholesterol, diabetes mellitus, and advancing age. The method involves assigning Framingham points for each major independent CHD risk factor. Framingham points are then summed and the RR for CHD is provided according to total Framingham points. Separate CHD RRs are provided for 5 year age brackets (30-74 for men; 40-47 for women). The Framingham Relative Risks were based on risk of CHD incidence over 10 years and are relative to the CHD incidence observed in the lowest risk strata in the cohort – defined as total serum cholesterol 160-199 mg/dl; LDL-C 100-129 mg/dl; HDL-C \geq 45 mg/dl in men or HDL-C \geq 55 mg/dl in women; BP <120 mmHg systolic and < 80 mmHg diastolic; non-smoker; and absence of diabetes mellitus.

One additional Framingham point is assigned for each category of SBP (i.e. 0 points for normal, 1 point for Pre-hypertension, 2 points for Stage I, and 3 points for Stage II). A 1% increase in population mean SBP will result in an additional prevalence of Pre, Stage I and Stage II Hypertension within the population. The additional prevalence of these respective stages of hypertension was earlier quantified via the normal distribution theory. Each of these additional cases of hypertension would experience a 1 point increase in their total Framingham point score. Therefore the increased RR for CHD associated with a 1 point increase in age and sex-specific total Framingham point score was determined.

The increased RR for CHD associated with a single point increase in total Framingham point score is variable depending on age, sex and the total Framingham point score for the other risk factors. For example; a change in total Framingham points from 7 to 8 points for a 44 year-old male is associated with an

increased RR for CHD of 1.0 (RR changes from 4.3 to 5.3), whereas a change in total Framingham points from 7 to 8 points for a 53 year-old female results in an increased RR for CHD of 0.2 (RR changes from 1.2 to 1.4). Therefore, the mean increase in CHD RR associated with a 1 point increase in total Framingham points was determined for each sex-specific decade age bracket included in the model. The mean increase in CHD RR associated for all risk strata as it was assumed that the 1% increase in population SBP would occur uniformly with respect to the distribution of underlying or additional CHD risk factors. This represents a departure from the methods of Equilibrium Environmental Inc. (2008a), where the increase in CHD RR was only calculated for a 1 point increase in total Framingham points for those at the bottom strata of CHD risk factors.

The model includes women 35-74 years old, but Framingham CHD RRs were only published for women 40-74 years old; therefore, the CHD RRs for women 40-44 years old were assumed to also apply to women 35-39 years old.

The average increase in RR of CHD for each sex-specific decade age-bracket (RR_{CDHi}) was used to calculate the incremental increase in CDH mortality resulting from the increased prevalence of hypertension associated with a 1% increase in population mean SBP. While Grundy *et al.* (1999) presented RRs for 10 year incidence of combined CHD morbidity and mortality, it was assumed, for the purpose of this report, that the proportion of people with CHD who died of CHD remained constant and therefore the Framingham RR for combined CHD morbidity and mortality.

Age and sex-corrected CHD mortality risks were age-standardized using the direct method and the 1997 Canadian Census population distribution (Health Canada, 1999).

First, the sex and age-adjusted additional prevalence of hypertension were calculated for each sex-specific decade age-bracket via Equation A9.

Equation A9

 $AG\Delta C_{H}(BMR)_{i} = \Delta C_{H}(BMR)_{i} \times AG_{i}$

Where

$$\Delta C_H (BMR)_i = \sum_{a=P}^{II} \Delta C_a (BMR)_i$$

and

 AG_i = The proportion of the total Canadian population between 35 and 75 years-old of the *i*th sex specific age-bracket.

Next, the sex and age-adjusted 10 year CHD mortality risks were calculated for each sex-specific decade age-bracket via Equation A10.

Equation A10

$$AGCHD_{M}(BMR)_{i} = \left[\left(\frac{M(0)_{i} \times RR_{CHDi}}{100,000} \right) - \left(\frac{M(0)_{i}}{100,000} \right) \right] \times AG\Delta C_{H}(BMR)_{i}$$

Where

 $M(0)_i$ = The baseline Canadian CHD 10-year mortality per 100,000

RR_{CHDi} = The Framingham Relative Risk for CHD

Finally, the sex and age-adjusted 10 year CHD mortality risks for each sex-specific decade age-bracket were summed to calculate a cumulative incremental CHD mortality per 100,000 for an adult lifespan (35-74 years) associated with a 1% increase in population mean SBP.

All model inputs, default parameters and outputs are presented in Tables A6-A8. A summary of the results is provided in Table A5. The total sex and age-adjusted additional prevalence of hypertension (Pre, Stage I and Stage II) among 35-74 yearolds associated with a 1% increase in population mean SBP is 5,421 per 100,000. The sex and age-adjusted cumulative (35-74 years) incremental risk of CHD mortality associated with a 1% increase in population mean SBP is 50 in 100,000. Males are much more sensitive that females and constitute about 80% of the modeled CHD deaths. A CHD mortality risk of 50 in 100,000 is 5 to 500 times the ILCR that is inherent in many Canadian occupational and environmental quality quidelines and standards (where the inherent ILCR is typically ranges from 1 in 10,000 to 1 in 1 million). The CHD mortality risk may also be considered qualitatively more significant, as ILCRs are based estimates of cancer potency that include nonlethal endpoints, such as incidence of pre-cancerous lesions. A 1% increase in SBP is, therefore, considered toxicologically relevant from a population health perspective and is an appropriate and defensible continuous Benchmark Response upon which to base a toxicological reference value for the prevention of unacceptable population health risks.

Assumptions, Uncertainties, and Limitations

Modeling is a simplification of an uncertain and variable reality. While there are important uncertainties and assumptions inherent in the models used here that limit their predictive accuracy, overall they are judged to be sufficiently accurate to provide a reasonable answer to the question at hand - which is whether the magnitude of population health effects associated with a 1% decrease in population IQ or a 1% increase in population SBP are toxicologically relevant and appropriate to use as a relative continuous Benchmark Response (BMR_c) for the derivation of a toxicological reference value. While refinements could be made to these models, it was felt the cumulative influence of the model uncertainties and assumptions identified below would not materially affect the overall conclusions of the modeling exercise.

The normal distribution theory used to calculate the increasing probability of MMR associated with decreasing population mean IQ and to calculate the increasing probability of hypertension with increasing population mean SBP assumes that the response (either IQ or SBP) is normally distributed with a constant variance. Deviations from these assumptions may affect the probability of the quantal adverse response (MMR or hypertension) in either direction. However, the expected effect in the context of this exercise is anticipated to be very small: population IQ is normally distributed by definition, Equilibrium Environmental Inc (2008a) reported that the normal distribution represents a reasonable approximation of the true distribution of national SBP data, and the standard deviations of both IQ and SBP are not expected to change significantly over the size of the shift in distribution that is modeled here (equivalent to a 1% change in mean).

Cumulative mortality risks were calculated for the age range of 35 to 74 years. Estimates of lifetime cancer risk based on Human Health Risk Assessment protocols in Canada typically consider an adult up to the age of 75 years. The exclusion of younger ages (< 35 years) in the calculation of SBP related CHD mortality risks has

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a minor impact on the quantitative estimates of CHD mortality risk because these life-stages have a relatively low base-line incidence of CHD mortality. The exclusion of older ages (75+ years) may underestimate risks of CHD mortality given the significant increase in baseline CHD mortality risk with increasing age, but baseline SBP data were not available for these ages. Additionally, the Framingham CHD mortality risk factors as a function of SBP may not be representative of those in the lower and higher extremities of age.

The models used to calculate the risk of hypertension and associated CHD mortality assume that any additional incidences of hypertension as a result of a 1% increase in SBP are not successfully treated. This assumption will result in an overestimation of the risks of hypertension and associated CHD mortality. However, the rates of hypertension diagnosis and treatment, relative to the prevalence of hypertension in Canada are quite low (Joffres et al. 1997). Additionally, the major independent risk factors for CHD are not necessarily elastic (i.e. commensurate reduction in CHD risk associated with reduction in risk factor) (Grundy et al. 1999) and treatment of hypertension may not entirely eliminate associated CHD risk. Therefore, this assumption is not expected to have a large impact on the magnitude or accuracy of the calculated risk estimates.

The model used to calculate the risk of CHD mortality assumes that the proportional incidence of CHD morbidity that results in CHD mortality remains constant (i.e the rate of success of case identification and successful intervention does not increase or decrease with increasing prevalence of CHD morbidity and therefore a 10% increase in the incidence of CHD will result in a commensurate 10% increase in CHD mortality). This assumption allows the application of the Framingham RR for combined CHD morbidity and mortality as a function of hypertension to be substituted for the RR of CHD mortality. This assumption was necessary to work with the available data; it also contributed to model parsimony. In the absence of

data to the contrary, it seems like a reasonable and defensible assumption in the context of the required model resolution.

The CHD mortality risks were derived from baseline Canadian CHD mortality from 1997. More recent (2003) CHD mortality data are now available and indicate a slight progressive decline in CHD mortality (HSFC 2006). Cumulative CHD mortality risks are linearly related to baseline CHD mortality, M(0) (see Equation A-10); therefore, to the extent that baseline mortality is overestimated, the cumulative CHD mortality risks will also be over-estimated. It is estimated that the decline in baseline Canadian CHD mortality would not affect the CHD risk estimates by more than 20% (HSFC 2006). An error of this magnitude would not change the overall conclusions of this modeling exercise.

Summary and Conclusions

The objectives of this section of the report were to:

Introduce the normal distribution theory. The normal distribution theory is a method of expressing a continuous endpoint, such as IQ or blood pressure, as a probability of exceeding a predefined cut-off on the continuous distribution that defines a quantal adverse outcome. e.g. a systolic blood pressure > 140 mmHg is defined as hypertension.

Describe the methods and results of application of the normal distribution theory to calculate the increased risk of Mild Mental Retardation (MMR) among children associated with a decrement in population mean IQ of 1%. The results of this modeling are that a 1% decrement in population mean IQ is associated with an added risk of MMR of approximately 1 in 250 (385 in 100,000) children.

Describe the methods and results of application of the normal distribution theory to calculate the increased probability of hypertension among adults 35-74 years old associated with an increase in population mean systolic blood pressure (SBP) of 1%. The total sex and age-adjusted added risk of hypertension (Pre, Stage I and Stage II) among 35-74 year-olds associated with a 1% increase in population mean SBP is approximately 1 in 20 (5,421 per 100,000).

Describe the methods and results of a Relative Risk model to calculate the *incremental cumulative risk of Coronary Heart Disease (CHD) mortality for adults 35-74 years old associated with an increase in population mean SBP of 1%.* The sex and age-adjusted cumulative (35-74 years) incremental risk of CHD mortality associated with a 1% increase in population mean SBP is 1 in 2,000 (50 per 100,000). Males are much more sensitive that females and constitute about 80% of the calculated CHD deaths

The conclusions are that a 1% change in population mean IQ and population mean SBP are both toxicologically significant from a population health perspective. The calculated population health risks associated with a 1% decrease in population mean IQ and a 1% increase in population mean SBP are at least a factor of 10 (and up to a factor of 1,000) greater than the calculated incremental lifetime cancer risks (ILCR) inherent in many environmental and occupational quality guidelines and standards. The uncertainties and variability in the calculated relationships and associated outputs are not of sufficient magnitude to affect the conclusions of the modeling exercise. Therefore, a relative continuous Benchmark Response (BMR_c) of 0.01 (1%) for these endpoints is appropriate and defensible for the derivation of

toxicological reference values for the prevention of unacceptable adverse population health effects due to environmental exposure to lead.

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Table A1. Added risk and extra risk of MMR and change in prevalence of MMR associated with decrements in various population mean IQ. The relative continuous Benchmark Response selected for derivation of Health Canada reference blood Pb concentrations is 1%.

Parameter	Units	Symbol	0.1% decrement in population IQ	1% decrement in population IQ	5% decrement in population IQ	10% decrement in population IQ	
Relative continuous BMR		BMRc	0.001	0.01	0.05	0.1	
Mean baseline population	IQ points	μ(0)	100	100	100	100	
Standard deviation of population IQ	IQ points	σ	15	15	15	15	
Cut-off on IQ distribution that defines MMR	IQ points	x0	70	70	70	70	
	1	T	1	1	1		
Baseline probability of MMR		P(0)	0.022750132	0.022750132	0.022750132	0.022750132	
Probability of MMR with 1% decrement in population mean IQ		P(BMRc)	0.023112479	0.026597574	0.047790352	0.09121122	
Added risk of MMR			3.62E-04	3.85E-03	2.50E-02	6.85E-02	
Extra risk of MMR			3.71E-04	3.94E-03	2.56E-02	7.01E-02	
Baseline prevalence of MMR	cases per 100,000		2,275	2,275	2,275	2,275	
Prevalence of MMR with 1% decrement in population mean IQ	cases per 100,000		2,311	2,660	4,779	9,121	
Increase in prevalence of MMR	cases per 100,000		36	385	2,504	6,846	

Age	Age SBP in MALES (mmHg)		SBP in FEMALES (mmHg)				
(years)	Mean	SD	Mean	SD			
18 – 24	121.6	10.8	111.2	9.5			
25 - 34	121.8	10.9	110.4	9.9			
35 – 44	123.4	12.4	114.1	12.1			
45 – 54	127.5	13.0	121.2	16.0			
55 – 64	137.3	17.0	133.1	17.5			
65 – 74	140.6	18.4	141.2	19.5			
75 – 84	139.9 (1)	19.2 (1)	141.0 (1)	17.1 (1)			

Table A2. Age and sex-specific distributions of systolic blood pressure in the Canadian population 1986-1992.

Source: Canadian Health Heart Database, 1992

SD – standard deviation

1 – data for Nova Scotia only

Table A3. Added risk and extra risk of Stage I Systolic Hypertension and added prevalence of Stage I Systolic Hypertension associated with a 1% increase in population mean systolic blood pressure. The added and extra risks are sex and age-specific, whereas the added prevalence of Stage I Systolic Hypertension are age corrected sex-specific.

rameter Units S		Symbol	Age						
Males			18-24	25-34	35-44	45-54	55-64	65-74	75-84
Baseline population mean SBP	mmHg	μ(0)	121.6	121.8	123.4	127.5	137.3	140.6	139.9
Baseline population standard deviation SBP	mmHg	σ	10.8	10.9	12.4	13	17	18.4	19.2
Relative continuous BMR		BMRc	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Population mean SBP @ BMR	mmHg	µ(BMR)	122.8	123.0	124.6	128.8	138.7	142.0	141.3
SBP cut-off for stage I hypertension	mmHg	x0I	140	140	140	140	140	140	140
Proportion of Canadian males 35-74	unitless	ঐAi	N/A	N/A	0.3786	0.291	0.1868	0.1435	N/A
Age-Specific ♂ Probability of Hypertension									
Baseline probability of stage I hypertension (SBP> x0I)	unitless	PI(0)	4.4E-02	4.7E-02	29.0E-02	21.7E-01	4.4E- 01	5.1E-01	5.0E-01
Probability of stage I hypertension (SBP> x0I) @ BMR	unitless	PI(BMR)	5.6E-02	6.0E-02	21.1E-0′	11.9E-01	4.7E- 01	5.4E-01	5.3E-01
Age-Specific ♂ Risk of Hypertension									
Added risk of stage I hypertension (SBP> x0I) @ BMR	unitless	N/A	1.2E-02	1.2E-02	21.7E-02	22.6E-02	3.2E- 02	3.0E-02	2.9E-02
Extra risk of stage I hypertension (SBP> x0I) @ BMR	unitless	N/A	1.2E-02	1.3E-02	21.9E-02	23.1E-02	5.7E- 02	6.2E-02	5.8E-02
Age-Specific ♂ Prevalence of Hypertension									
Baseline prevalence of stage I hypertension (SBP> x0I)	per 100,000 age bracket	CI(0)	4,422	4,749	9,033	16,814	43,690	51,301	49,792
Prevalence of stage I hypertension (SBP> x0I) @ BMR	per 100,000 age bracket	CI(BMR)	5,579	5,962	10,764	19,394	46,889	54,341	52,697
Additional prevalence of stage I hypertension @ BMR	per 100,000 age bracket	ΔCI(BMR)	1,157	1,213	1,731	2,580	3,199	3,040	2,905
Age-Adjusted ♂ Prevalence of Hypertension									Σ
Age-adjusted additional prevalence of stage I hypertension @ BMR	oper 100,000 35- 74yrs	AΔCI(BMR)	N/A	N/A	655	751	598	436	2,440

Risk Factor	Age-Specific Mortality per 100,000							
	35-44	45-54	55-64	65-74	75-84	85+		
MALES								
IHD	19 (0.019%)	78 (0.078%)	267 (0.27%)	702 (0.70%)	1825 (1.8%)	4020 (4.0%)	6911 (6.9%)	
AMI	11	45	161	385	924	1662	3188	
CEVD	4	11	37	159	568	1639	2418	
Other CVD	8	19	71	231	714	2122	3165	
All CVD	31	109	376	1092	3107	7781	12496	
FEMALES								
IHD	4 (0.004%)	19 (0.019%)	85 (0.085%)	296 (0.30%)	1029 (1.0%)	3162 (3.2%)	4595 (4.6%)	
AMI	2	11	51	173	542 1251		2030	
CEVD	3	10	28	103	450	1688	2282	
Other CVD	4	10	36	129	477	2099	2755	
All CVD	11	39	149	528	1956	6950	9633	

Table A4. Canadian age and sex-specific mortality from cardiovascular disease in 1997.

Appendix A

Table A5. Age and sex adjusted cumulative additional prevalence of hypertension and cumulative CHD mortality risk for ages 35-74 years-old associated with a 1% increase in population mean SBP.

Parameter	Units	Symbol	Age				
Males & Females			35-44	45-54	55-64	65-74	Σ
♂ Proportion of Canadians 35- 73	per 100,000 35-74yrs	Ç AG i	0.18715	0.14383	0.09235	0.07094	
$\stackrel{\circ}{_{ m Proportion}}$ Proportion of Canadians 35-74	per 100,000 35-74yrs	ੰAGi	0.18571	0.14296	0.09459	0.08247	
් Age and Gender-Adjusted additional prevalence hypertension	per 100,000 35-74yrs	∂AG∆CH(BMR)	996	956	691	524	3,166
♀ Age and Gender-Adjusted additional prevalence hypertension	per 100,000 35-74yrs	⊊ AGΔCH(BMR)	392	630	643	590	2,255
sum	per 100,000 35-74yrs	ΣΑGΔCH(BMR)	1,388	1,586	1,334	1,114	5,422
ঁ Age and Gender-Adjusted additional CHD mortality	per 100,000 35-74yrs	∂AG∆CHDM(BMR)	2	7	12	18	39
♀ Age and Gender-Adjusted additional CHD mortality	per 100,000 35-74yrs	⊊ AG∆CHDM(BMR)	0	1	2	8	11
sum	per 100,000 35-74yrs	ΣAGΔCHDM(BMR)	2	8	14	26	50

Source: HSFC (1999) – digitized from Figures 3-12 and 3-13 (HSFC, 1999)

CVD - cardiovascular diseases; IHD - ischemic heart disease (% incidence in brackets); CEVD – cerebrovascular diseases (includes stroke); AMI - acute myocardial infarction (heart attack) - a sub-category of IHD and not added

Baseline population standard deviation SBP	mmHa	σ	10.8	10.9	12.4	13	17	18.4	19.2	Canadian Health Heart Database, 1992
Relative continuous BMR	<u> </u>	BMRc	0.01	0.01	0.01	0.01	0.01	0.01	0.01	Input
Population mean SBP @ BMR	mmHg	µ(BMR)	122.8	123.0	124.6	128.8	138.7	142.0	141.3	Calculated
SBP cut-off for pre-hypertension	mmHg	x0P	130	130	130	130	130	130	130	Grundy et al., 1999
SBP cut-off for stage I hypertension	mmHg	x0I	140	140	140	140	140	140	140	Grundy et al., 1999
SBP cut-off for stage II hypertension	mmHg	x0II	160	160	160	160	160	160	160	Grundy et al., 1999
Baseline age-specific CHD mortality	per 100,000 per 10 yrs	M(0)	N/A	N/A	190	780	2670	7020	N/A	Heart & Stroke Foundation Canada, 1999
Relative Risk of CHD mortality	unitless	RRCHD	N/A	N/A	2.28	1.95	1.64	1.49	N/A	Grundy et al., 1999
Proportion of Canadian males 35-74	unitless	ੈAi	N/A	N/A	0.3786	0.291	0.1868	0.1435	N/A	Health Canada, 1999
Age-Specific 👌 Probability of Hypertension										
Baseline probability of pre-hypertension (SBP> x0P)	unitless	PP(0)	2.2E-01	2.3E-01	3.0E-01	4.2E-01	6.7E-01	7.2E-01	7.0E-01	Calculated
Baseline probability of stage I hypertension (SBP> x0I)	unitless	PI(0)	4.4E-02	4.7E-02	9.0E-02	1.7E-01	4.4E-01	5.1E-01	5.0E-01	Calculated
Baseline probability of stage II hypertension (SBP> x0II)	unitless	PII(0)	1.9E-04	2.3E-04	1.6E-03	6.2E-03	9.1E-02	1.5E-01	1.5E-01	Calculated
Probability of pre-hypertension (SBP> x0P) @ BMR	unitless	PP(BMR)	2.5E-01	2.6E-01	3.3E-01	4.6E-01	7.0E-01	7.4E-01	7.2E-01	Calculated
Probability of stage I hypertension (SBP> x0I) @ BMR	unitless	PI(BMR)	5.6E-02	6.0E-02	1.1E-01	1.9E-01	4.7E-01	5.4E-01	5.3E-01	Calculated
Probability of stage II hypertension (SBP> x0II) @ BMR	unitless	PII(BMR)	2.9E-04	3.5E-04	2.2E-03	8.2E-03	1.0E-01	1.6E-01	1.7E-01	Calculated
Age-Specific 🖒 Prevalence of Hypertension										
Baseline prevalence of pre-hypertension (SBP> x0P)	per 100,000 age bracket	CP(0)	21,835	22,594	29,727	42,375	66,619	71,772	69,694	Calculated
Baseline prevalence of stage I hypertension (SBP> x0I)	per 100,000 age bracket	Cl(0)	4,422	4,749	9,033	16,814	43,690	51,301	49,792	Calculated
Baseline prevalence of stage II hypertension (SBP> x0II)	per 100,000 age bracket	CII(0)	19	23	158	621	9,089	14,586	14,758	Calculated
Prevalence of pre-hypertension (SBP> x0P) @ BMR	per 100,000 age bracket	CP(BMR)	25,297	26,091	33,260	46,246	69,504	74,296	72,190	Calculated
Prevalence of stage I hypertension (SBP> x0I) @ BMR	per 100,000 age bracket	CI(BMR)	5,579	5,962	10,764	19,394	46,889	54,341	52,697	Calculated
Prevalence of stage II hypertension (SBP> x0II) @ BMR	per 100,000 age bracket	CII(BMR)	29	35	217	815	10,482	16,405	16,503	Calculated
Additional prevalence of pre-hypertension @ BMR	per 100,000 age bracket	ΔCP(BMR)	3,462	3,497	3,533	3,871	2,885	2,524	2,496	Calculated
Additional prevalence of stage I hypertension @ BMR	per 100,000 age bracket	ΔCI(BMR)	1,157	1,213	1,730	2,580	3,199	3,040	2,905	Calculated
Additional prevalence of stage II hypertension @ BMR	per 100,000 age bracket	ΔCII(BMR)	10	12	59	194	1,393	1,819	1,745	Calculated
Age-Adjusted ♂ Prevalence of Hypertension									Σ	
Age-adjusted additional prevalence of pre-hypertension @ BMR	per 100,000 35-74yrs	A∆CP(BMR)	N/A	N/A	1,338	1,126	539	362		Calculated
Age-adjusted additional prevalence of stage I hypertension @ BMR	per 100,000 35-74yrs	A∆CI(BMR)	N/A	N/A	655	751	598	436		Calculated
Age-adjusted additional prevalence of stage II hypertension @ BMR	per 100,000 35-74yrs	A∆CII(BMR)	N/A	N/A	22	56	260	261		Calculated
Total	per 100,000 35-74yrs				2,015	1,933	1,397	1,059	6,404	Calculated
Age-Adjusted ♂ CHD Mortality										
Age-adjusted additional CHD mortality @ BMR	per 100,000 35-74yrs				5	14	24	36	79	Calculated

Table A6. Model defaults, inputs and outputs for calculation of age adjusted cumulative CHD mortality risk per 100,000 for males 35-74 years old associated with a 1% increase in population mean systolic blood pressure.

Parameter	Units	Symbol	Age							Source
Females			18-24	25-34	35-44	45-54	55-64	65-74	75-84	
Baseline population mean SBP	mmHg	μ(0)	111.2	110.4	114.1	121.2	133.1	141.2	141	Canadian Health Heart Database, 1992
Baseline population standard deviation SBP	mmHg	σ	9.5	9.9	12.1	16	17.5	19.5	17.1	Canadian Health Heart Database, 1992
Relative continuous BMR		BMRc	0.01	0.01	0.01	0.01	0.01	0.01	0.01	Input
Population mean SBP @ BMR	mmHg	µ(BMR)	112.3	111.5	115.2	122.4	134.4	142.6	142.4	Calculated
SBP cut-off for pre-hypertension	mmHg	x0P	130	130	130	130	130	130	130	Grundy et al., 1999
SBP cut-off for stage I hypertension	mmHg	x0I	140	140	140	140	140	140	140	Grundy et al., 1999
SBP cut-off for stage II hypertension	mmHg	x0II	160	160	160	160	160	160	160	Grundy et al., 1999
Baseline age-specific CHD mortality	per 100,000 per 10 yrs	M(0)	N/A	N/A	40	190	850	3000	N/A	HSFC, 1999
Relative Risk of CHD mortality	unitless	RRCHD	N/A	N/A	1.74	1.48	1.43	1.43	N/A	Grundy et al., 1999
Proportion of Canadian females 35-74	unitless	ÇAi	N/A	N/A	0.3672	0.2827	0.187	0.1631	N/A	Health Canada, 1999
Age-Specific $\[Therefore]$ Probability of Hypertension										
Baseline probability of pre-hypertension (SBP> x0P)	unitless	PP(0)	2.4E-02	2.4E-02	9.4E-02	2.9E-01	5.7E-01	7.2E-01	7.4E-01	Calculated
Baseline probability of stage I hypertension (SBP> x0I)	unitless	PI(0)	1.2E-03	1.4E-03	1.6E-02	1.2E-01	3.5E-01	5.2E-01	5.2E-01	Calculated
Baseline probability of stage II hypertension (SBP> x0II)	unitless	PII(0)	1.4E-07	2.7E-07	7.4E-05	7.7E-03	6.2E-02	1.7E-01	1.3E-01	Calculated
Probability of pre-hypertension (SBP> x0P) @ BMR	unitless	PP(BMR)	3.1E-02	3.1E-02	1.1E-01	3.2E-01	6.0E-01	7.4E-01	7.7E-01	Calculated
Probability of stage I hypertension (SBP> x0I) @ BMR	unitless	PI(BMR)	1.8E-03	2.0E-03	2.0E-02	1.4E-01	3.8E-01	5.5E-01	5.6E-01	Calculated
Probability of stage II hypertension (SBP> x0II) @ BMR	unitless	PII(BMR)	2.6E-07	4.8E-07	1.1E-04	9.4E-03	7.2E-02	1.9E-01	1.5E-01	Calculated
Age-Specific $\[Prevalence of Hypertension$										
Baseline prevalence of pre-hypertension (SBP> x0P)	per 100,000 age bracket	CP(0)	2,391	2,386	9,441	29,116	57,030	71,714	73,998	Calculated
Baseline prevalence of stage I hypertension (SBP> x0I)	per 100,000 age bracket	Cl(0)	122	140	1,616	12,000	34,669	52,453	52,332	Calculated
Baseline prevalence of stage II hypertension (SBP> x0II)	per 100,000 age bracket	CII(0)	0	0	7	765	6,213	16,750	13,326	Calculated
Prevalence of pre-hypertension (SBP> x0P) @ BMR	per 100,000 age bracket	CP(BMR)	3,131	3,086	11,128	31,766	59,994	74,111	76,600	Calculated
Prevalence of stage I hypertension (SBP> x0I) @ BMR	per 100,000 age bracket	CI(BMR)	178	200	2,037	13,583	37,516	55,328	55,604	Calculated
Prevalence of stage II hypertension (SBP> x0II) @ BMR	per 100,000 age bracket	CII(BMR)	0	0	11	941	7,200	18,628	15,182	Calculated
Additional prevalence of pre-hypertension @ BMR	per 100,000 age bracket	ΔCP(BMR)	740	700	1,687	2,650	2,964	2,397	2,602	Calculated
Additional prevalence of stage I hypertension @ BMR	per 100,000 age bracket	ΔCI(BMR)	56	60	421	1,583	2,847	2,875	3,272	Calculated
Additional prevalence of stage II hypertension @ BMR	per 100,000 age bracket	ΔCII(BMR)	0	0	4	176	987	1,878	1,856	Calculated
Age-Adjusted $\[Therefore]$ Prevalence of Hypertension									Σ	
Age-adjusted additional prevalence of pre-hypertension @ BMR	per 100,000 35-74yrs	AΔCP(BMR)	N/A	N/A	619	749	554	391		Calculated
Age-adjusted additional prevalence of stage I hypertension @ BMR	per 100,000 35-74yrs	AΔCI(BMR)	N/A	N/A	155	448	532	469		Calculated
Age-adjusted additional prevalence of stage II hypertension @ BMR	per 100,000 35-74yrs	AΔCII(BMR)	N/A	N/A	1	50	185	306		Calculated
Total	per 100,000 35-74yrs				775	1,247	1,270	1,166	4,459	Calculated
Age-Adjusted ♀ CHD Mortality	· · · · · · · · · · · · · · · · · · ·								Σ	
Age-adjusted additional CHD mortality @ BMR	per 100,000 35-74yrs				0	1	5	15	21	Calculated

Table A7. Model defaults, inputs and outputs for calculation of age adjusted cumulative CHD mortality risk per 100,000 for females 35-74 years old associated with a 1% increase in population mean systolic blood pressure.
Table A8. Model defaults, inputs and outputs for calculation of age and sex adjusted cumulative CHD mortality risk per 100,000 (35-74 years old) associated with a 1% increase in population mean systolic blood pressure.

Parameter	Units	Symbol				Age				Source
Males & Females			18-24	25-34	35-44	45-54	55-64	65-74	75-84	-
Age & Gender-Adjusted CHD Mortality									Σ	
් Proportion of Canadians 35-73	per 100,000 35-74yrs	ÇAGi			0.18715	0.14383	0.09235	0.07094		Calculated
♀ Proportion of Canadians 35-74	per 100,000 35-74yrs	ੈAGi			0.18571	0.14296	0.09459	0.08247		Calculated
∂ Age and Gender-Adjusted additional prevalence hypertension	per 100,000 35-74yrs	്AG∆CH(BMR)			996	956	691	524		Calculated
♀ Age and Gender-Adjusted additional prevalence hypertension	per 100,000 35-74yrs	ୁAG∆CH(BMR)			392	630	643	590	J	Calculated
sum	per 100,000 35-74yrs	ΣAGΔCH(BMR)			1388	1586	1334	1114	5422	Calculated
් Age and Gender-Adjusted additional CHD mortality	per 100,000 35-74yrs	്AG∆CHDM(BMR)			2	7	12	18		Calculated
♀ Age and Gender-Adjusted additional CHD mortality	per 100,000 35-74yrs	⊊AG∆CHDM(BMR)			0	1	2	8		Calculated
sum	per 100,000 35-74yrs	ΣAGΔCHDM(BMR)			2	8	14	26	50	Calculated

APPENDIX B

Summary Tables of 12 Prospective Cohort Studies of

Early Life Blood Lead and Children's IQ

Boston, MA USA				Pb exposure index;	cord	cord								mean		
				unless otherwise	blood <	blood 6-	cord blood							cord - 5	mean 0.5 -	mean 1 - 5
				notod	2 ug/dl	Z ug/dl	> 10 ug/dl	0.5.100	1.10	1.5.400	2.100	E viro	10	0010 - 0	5 vrc	incarr - o
-				Mean	1.8	6.5	14.6	NR	NK	NR	7.0	6.4	2.9	NR		NR
				SD	0.6	0.3	3.0	NR	NR	NR	6.6	4.1	2.4	NR	NR	NR
				min	NR	NR	NR	NR	NR	NR	NR	0	0.5	NR	NR	NR
Reference	Age	Endpoint	n	max	NR	NR	NR	NR	NR	NR	23.6	23.3	16	NR	NR	NR
Bellinger et al. (1984)	0.5	BSMD MDI	201	Adjusted MDI scores	110.2	108.0	105.9	-	-	-	-	-	-	-	-	-
Bellinger et al. (1986)	1	BSMD MDI	199	Adjusted MDI scores	114.7	114.4	108.9	-	-	-	-	-	-	-	-	-
Bellinger et al. (1987)	1.5	BSMD MDI	182	Adjusted MDI scores	116.2	114.8	109.5	-	-	-	-	-	-	-	-	-
Bellinger et al. (1987)	2	BSMD MDI	182	Adjusted MDI scores	118.9	117.8	111.1	-	-	-	-	-	-	-	-	-
Bellinger et al. (1991)	5	MSCA GCI	170	Adjusted ß(Ln BPb)	-	-	-	0.28	-1.43	-1.62	-2.95	-2.28	-	-2.76	-2.33	-2.17
Bellinger et al. (1992)	10	IQ (WISC-R)	148	Adjusted ß	-	-	-	-0.13	0.00	-0.12	-0.58	-0.26	-0.46	-	-	-
		Shaded cells =	statis =	tically significant associa	ation (p<0	.05, 2-side	ed)									

				Pb exposure index;	materna											
				blood Pb (µg/dL)	I								mean			
				unless otherwise	prenant		neonatal		mean 1			mean 4	birth - 4	mean 5	mean birth ·	
Cincinnati Lead Study				noted	al	cord	(10 day)	3 month	yr	mean 2 yr	mean 3 yr	yr	yrs	yr	5 yrs	mean 6 yr
				Mean	8	6.3	4.6	5.9	10.6	17.2	16.3	14.1	NR	11.9	NR	NR
				SD	3.7	4.5	2.8	3.4	5.1	8.4	7.8	7.3	NR	6.4	NR	NR
				min	1	1	1	1	3	6	4	3	NR	3	NR	NR
Reference	Age	Endpoint	n	max	27	28	22	22	35	49	50	45	NR	38	NR	NR
Dietrich et al. (1987)	0.25	Bayley MDI	249	Adjusted ß	-0.34	-0.6	0.06	-0.23	-	-	-	-	-	-	-	-
Dietrich et al. (1987)	0.5	Bayley MDI	249	Adjusted ß	-0.76	-0.66	-3.49	-0.48		-	-	-	-	-	-	-
Dietrich et al. (1990)	2	Bayley MDI	297	Adjusted ß	0.5058	-	-0.0162	-	-	0.127	-	-	-	-	-	-
Dietrich et al. (1991)	4	K-ABC MPC	247	Adjusted ß	0.11	-	-0.91 ¹	-	-0.03	-0.02	-0.08	-0.1	-0.07	-	-	-
Dietrich et al. (1992)	5	K-ABC MPC	259	Adjusted ß	0.22	-	-0.1	-	0.07	0.02	-0.06	-0.12	-	-0.13	-0.06	-
Dietrich et al. (1993)	6.5	IQ (WISC-R)	253	Adjusted ß	0.15	-	-0.03	-	0.01	-0.02	-0.13	-0.15	-	-0.23	-0.13	-0.33
Ris et al. (2004)	15-17	factor	195	Adjusted ß	-0.075							-			-0.34	

Additional and the second sec

Boston, MA USA				Pb exposure index;											
				blood Pb (µg/dL)				Dentine							
				unless otherwise	mean 1.5 -	mean 2 -	mean 2 -	Pb							
				noted	5 yrs	5 yrs	10 yrs	(µq/q)						Other covariates	Exclusion criteria
				Mean	NR	NR	NR	2.8	Maternal						
				SD	NR	NR	NR	1.7	drug,		Quality of			material and 10 method status	
				min	NR	NR	NR	0.3	alcohol &		Caregiver	Maternal	Subject nutritional	maternal age, IQ, martital status,	
Reference	Age	Endpoint	n	max	NR	NR	NR	12.8	tobaco use	SES	Environment	IQ	status	education, gravidity, parity, history of	Down's syndrome, cleft palate,
Bellinger et al. (1984)	0.5	BSMD MDI	201	Adjusted MDI scores	-	-	-	-	✓	~	~	~		 miscarriage, obstetrical complications, 	gestational age < 34 weeks, and
Bellinger et al. (1986)	1	BSMD MDI	199	Adjusted MDI scores	-	-	-	-	✓	~	~	~		 and smoking and alconol consumption ; 	retinoblastoma and non-English
Bellinger et al. (1987)	1.5	BSMD MDI	182	Adjusted MDI scores	-	-	-	-	✓	~	~	~		 parental education and social class; 	speaking
Bellinger et al. (1987)	2	BSMD MDI	182	Adjusted MDI scores	-	-	-	-	✓	~	~	~		- subject sex, 5 minute Apgar, birth weight,	
Bellinger et al. (1991)	5	MSCA GCI	170	Adjusted ß(Ln BPb)	-2.94	-2.80	-	-2.51	✓	~	~	~	1	 HOME environment, and 	
Bellinger et al. (1992)	10	IQ (WISC-R)	148	Adjusted ß	-	-0.82	-0.86		✓	~	✓	~	✓		
		Shaded cells =	= statis	tically significant associ	a										

Cincinnati Lead Study				Pb exposure index; blood Pb (µg/dL) unless otherwise noted	mean birth - 6 yrs	78 month						Other covariates	Exclusion criteria
				Mean	ŃR	NR	Maternal						
				SD	NR	NR	drug,		Quality of	Material	Outlined autobiline of		
				min	NR	~5	alconol &		Caregiver	Maternal	Subject nutritional	Maternal age at birth, obstetrical	mother's addicted to drugs or alcohol.
Reference	Age	Endpoint	n	max	NR	~15	tobaco use	SES	Environment	IQ	status	- complications score signature alcohol	with diabates or neurological
Dietrich et al. (1987)	0.25	Bayley MDI	249	Adjusted ß	-	-	✓	~	~	~	✓	and drug use during pregnancy, history of	disorders psychoses or mental
Dietrich et al. (1987)	0.5	Bayley MDI	249	Adjusted ß	-	-	✓	~	~	~	✓	- and drug use during pregnancy, history of	retardation and subjects < 25 wooks
Dietrich et al. (1990)	2	Bayley MDI	297	Adjusted ß	-	-	✓	~	~	~	1	 abortion and stillbirths, gravidity, parity, IO: subject birth weight birth length 	destation < 1.500 a birth weight or
Dietrich et al. (1991)	4	K-ABC MPC	247	Adjusted ß	-	-	✓	~	✓	~	✓	neonatal head circumference, gestational	genetic syndromes or other serious
Dietrich et al. (1992)	5	K-ABC MPC	259	Adjusted ß	-	-	✓	~	✓	~	✓	age, 1 & 5 m	medical conditions at birth
Dietrich et al. (1993)	6.5	IQ (WISC-R)	253	Adjusted ß	-	-	✓	~	~	~	✓		
Ris et al. (2004)	15-17	factor	195	Adjusted ß	-	-0.81	1	~	1	~	~		

Shaded cells = statistically significant associa 1. Association significant, but for positive correlation b

issociation significant, but for positive correlation c

Cleveland, OH USA				Pb exposure index;									
				blood Pb (µg/dL)	materna						circumpulpal		
				unless otherwise	1@					mean	dentine Pb		
				noted	delivery	cord	6 month	2 yrs	3 yrs	preschool	µg/g		
				Mean	6.50	5.89	9.99	16.70	16.70	NR	63.4		
				SD	1.84	2.10	3.32	6.45	6.01	NR	71.71		
				min	2.7	2.8	5.0	5.4	6.4	NR	NR		
Reference	Age	Endpoint	n	max	11.8	14.7	24.0	41.8	42	NR	NR		
Ernhart et al. (1987)	0.5	BSMD MDI	127	Δr	0.0302	0.0046	0.0003	-		-	-		
Ernhart et al. (1987)	0.5	BSMD PDI	127	Δr	0.0295	0.0006	0.0084	-	-	-	-		
Ernhart et al. (1987)	0.5	KID	119	Δr	0.0779	0.0107	0.0692	-	-	-	-		
Ernhart et al. (1987)	1	BSMD MDI	145	Δr	0.0002	0.0146	0.0032	-	-	-	-		
Ernhart et al. (1987)	2	BSMD MDI	142	Δr	0.0020	0.0003	0.0007	0.0006	-	-	-		
Ernhart et al. (1987)	3	IQ (S-BIS)	138	Δr	0.0059	0.0125	0.0065	0.0087	0.0001	-	-		
Ernhart et al. (1989)	4.8	IQ (WPPSI)	212	Δr	0.0008	0.0008	0.0000	0.0058	0.0000	-	-		
Greene et al. (1993)	4.8	IQ (WPPSI)	164	Adjusted ß(sqrt Pb)	-	-	-	-	-	-	-0.6019		
		Shaded cells =	= statis	tically significant associa	ation (p<0.	05, 2-side	d)						
	2. Ass	ociation significa	ant for	lower SES stratum only									

Lead and Fetal Neurodev City, Mexico	elopme	ent Study, Mexi	co	Pb exposure index; blood Pb (µg/dL) unless otherwise noted	cord	maternal tibia Pb	maternal patella Pb	1 yr	2yr	
				mean	6.7	11.5	17.9	7.2	8.4	
				SD	3.4	11.0	15.2	2.8	4.6	
				min	1.2	<1	<1	2.3	2.5	
Reference	Age	Endpoint	n	max	21.6	85.9	76.6	18.9	38.6	
				ßs for patella Pb			-5.36 to -			
Gomaa et al. (2002)	2	Bayley MDI	197	quartile	-4.48	not sig	7.21	not sig	not sig	
		Shaded cells =	= statis	tically significant associa	ition (p<0	0.05, 2-side	d)			

Mexico City Prospective	Lead S	tudy		Pb exposure index;	materna	maternal										
				blood Pb (µg/dL)	l 12-20	28-36	maternal			mean 0.5 -		mean 1-	mean 2 -			mean 3.5 -
				unless otherwise	weeks	weeks	delivery	cord	1 yr	1.5 yrs	2yr	2yrs	3 yrs	3yr	4yr	4.5 yrs
				Geo mean	8.2	7.8	NR	NR	10.8	10.1	12.8	NR	9.7	11.3	10.3	8.4
				SD	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
				5th %ile	3.0	2.5	NR	NR	4.0	NR	5.0	NR	NR	4.7	4.2	NR
Reference	Age	Endpoint	n	95th %ile	20.7	24.6	NR	NR	22.0	NR	25.8	NR	NR	22.9	20.5	NR
Schnaas et al. (2000)	3-5	MSCA _S GCI	112	Adjusted ß(In BPb)	not sig	not sig	not sig	not sig	_	not sig	-	-	negative @ 48 month GCI	-	-	negative @ 54 month GCI
Schnaas et al. (2006)	6-10	IQ (WISC-R _s)	150	Adjusted ß(In BPb)	-1.45	-3.90	-1.29	-0.95	0.51	-	0.91	0.6	-	-0.58	1.17	-
		Shaded cells =	statis	tically significant asso	ciation (p<0	.05, 2-side	d)									

3. HOME Inventory measured during pregnancy or within 6 months postpartum

2 Cohort Study, Mexico	City, Me	exico		blood Pb (µg/dL)	cord	1. ver	2.11
				uniess ounerwise	4.05	1 91	2 1
				mean	4.85	4.27	4.28
				SD	3.00	2.14	2.25
				min	NR	NR	NR
Reference	Age	Endpoint	n	max	NR	NR	NR
Téllez-Rojo et al. (2006)	1	Bayley MDI	294	Adjusted ß(In BPb)	-	-1.14	-
Téllez-Rojo et al. (2006)	2	Bayley MDI	294	Adjusted ß(In BPb)	-	-	-4
		Shadod collo	- ctotic	tically cignificant accord	ation (n =0	OF 2 cido	4)

Shaded cells = statistically significant association (p<0.05, 2-sided)

Cleveland, OH USA				Pb exposure index; blood Pb (µg/dL) unless otherwise						0.1	European editoria
Reference	Age	Endpoint	n	noted Mean SD min max	Maternal drug, alcohol & tobaco use	SES	Quality of Caregiver	Maternal	Subject nutritional	included maternal IQ, alcohol, smoking	Exclusion criteria
Ernhart et al. (1987)	0.5	BSMD MDI	127	Δr	√	~	√	~	otatuo	 and drug use; parental education, 	< 37 weeks gestation, neonatal
Ernhart et al. (1987)	0.5	BSMD PDI	127	Δr	✓	√	~	~		 Authoritarian Family Ideology (AFI); and 	intensive care, maternal use of
Ernhart et al. (1987)	0.5	KID	119	Δr	✓	~	~	~		 subject sex, age at testing, birth order, attrainity, home and preached LIOME 	narcotics, English as a second
Ernhart et al. (1987)	1	BSMD MDI	145	Δr	✓	~	~	~		 ethnicity, nome and preschool HOME 	language, and maternal
Ernhart et al. (1987)	2	BSMD MDI	142	Δr	✓	~	~	~		 Inventory scores, preschool period 	schizophrenia.
Ernhart et al. (1987)	3	IQ (S-BIS)	138	Δr	✓	~	~	~		medical history and psycholog	
Ernhart et al. (1989)	4.8	IQ (WPPSI)	212	Δr	✓	~	~	1			
Greene et al. (1993)	4.8	IQ (WPPSI)	164	Adjusted ß(sqrt Pb)	✓	~	~	~			

Shaded cells = statistically significant associa 2. Association significant for lower SES stratum only

Lead and Fetal Neuroe	developm	ent Study, Mex	со	Pb exposure index;							
City, Mexico		-		blood Pb (µg/dL)							
				unless otherwise							
				noted	Mater	al				Other covariates	Exclusion criteria
				mean	drug						
				SD	alcoh	bl		Quality of	Subject	maternal age, IQ, education, and marital status;	
				min	& toba	00		Caregiver	nutritional	paternal education; and infant gender, gestational	extensive list of relevant medical conditions, intention not to breastfeed,
Reference	Age	Endpoint	n	max	use	S	SES	Environment Maternal IQ	status	age, medical history and duration of	gestational age < 37 weeks, 5 minute Apgar < 6, or birth weight < 2000 g
				ßs for patella Pb						breastfeeding	
Gomaa et al. (2002)	2	Bayley MDI	197	quartile				~			

Shaded cells = statistically significant associa

Mexico City Prospectiv	e Lead S	Study		Pb exposure index;											
				blood Pb (µg/dL)		mean 1-	mean 3-	mean 6-							
				unless otherwise	5yr	5yrs	5yrs	10yrs						Other covariates	Exclusion criteria
				Geo mean	9.3	9.8	NR	6.2	Maternal						
				SD	NR	NR	NR	NR	drug,		Quality of				
				5th %ile	3.8	2.8	NR	2.2	alcohol &		Caregiver	Maternal	Subject nutritional		
Reference	Age	Endpoint	n	95th %ile	18.0	36.4	NR	18.6	tobacco use SE	3	Environment	IQ	status	Outlinet and blath under the first IO	<36 weeks gestational age, 5 min
														Subject sex, birth weight, first IQ measurement, 5 min Agpar score	Agpar < 6, birth weight < 2000 g, congenital anomalies
Schnaas et al. (2000)	3-5	MSCA _S GCI	112	Adjusted ß(In BPb)	-	-	-	-	~			~			
Schnaas et al. (2006)	6-10	IQ (WISC-R _s)	150	Adjusted ß(In BPb)	-0.32	0.49	-0.08	-2.45	✓		✓ ³	~			
		Shaded cells =	statis	tically significant associa											
	2 10	ME Inventory me	ocuro	d during programmy or wit											

HOME Inventory measured during pregnancy or wit

2 Cohort Study, Mexico C	City, Me	exico		blood Pb (µg/dL)							
				unless otherwise	Maternal					Other covariates	Exclusion criteria
				mean SD min	drug, alcohol & tobaco		Quality of Caregiver		Subject nutritional	maternal age, IQ, education, and marital status; paternal education; and infant gender, gestational	extensive list of relevant medical conditions, intention not to breastfeed,
Reference	Age	Endpoint	n	max	use	SES	Environment	Maternal IQ	status	age, medical history and duration of	gestational age < 37 weeks, 5 minute Apgar < 6, or birth weight < 2000 g
Téllez-Rojo et al. (2006)	1	Bayley MDI	294	Adjusted ß(In BPb)				~		breastfeeding	
Téllez-Rojo et al. (2006)	2	Bayley MDI	294	Adjusted ß(In BPb)				~			
		Shaded cells	= statis	tically significant associa							

Port Pirie, Australia				Pb exposure index;												
				blood Pb (µg/dL)	mean					mean 0.5-2		mean 0-		mean 0-	AUC 0.5-4	
				unless otherwise	prenatal	cord	6 mo	15 month	2 yr	yr	3yr	3 yr	4 yr	4 yr	yrs	5 yr
				Geo mean	9.5	8.5	14.4	20.9	21.2	18.1	19.3	NR	NR	NR	NR	14.3
				SD	1.2	1.7	NR	1.4	NR	NR	1.2	NR	NR	NR	NR	1.4
				min	3.7	0.8	NR	5.2	2	NR	6.4	NR	NR	NR	NR	4.8
Reference	Age	Endpoint	n	max	33.1	36.6	NR	51.8	67	NR	62.1	NR	NR	NR	NR	40.8
Wigg et al. (1988)	2	BSMD MDI	595	Adjusted ß	-0.07	0.10	-0.16	-0.03	-0.05	-0.12						
McMichael et al. (1988)	4	MSCA GCI	537	Adjusted ß(Log BPb)	-0.4	3.3	-8.5	-3.5	-11.1		-13.1	· ·	-5.5	-	-15	
Baghurst et al. (1992)	7	IQ (WISC-R)	494	Adjusted ß(Log BPb)	-1.4	0.6	-	-	-	-4.6	-	-4.8	-	-4.6	-	-
Tong et al.(1996)	11-13	IQ (WISC-R)	375	Adjusted ß(Log BPb)		1.2		-1.8	-		-3.8	-4.3	-	-		-4
		Shaded cells =	= statis	tically significant associ	ation (p<0.	05, 2-side	d)									

Sydney, Australia				Pb exposure index;											
				blood Pb (µg/dL)										mean	
				unless otherwise	prenatal		mean 0-1	mean 1-2	mean 2-3	mean 3-4	mean 0-4	mean 3-		0.5 -7	
				noted	(at birth)	cord	yrs	yrs	yrs	yrs	yrs	5 yrs	7 yr	yrs	
				Mean	9.1	8.1	NR	NR	NR	NR	NR	NR	7.7	NR	
				SD	1.3	1.4	NR	NR	NR	NR	NR	NR	NR	NR	
				min	3	1	NR	NR	NR	NR	NR	NR	NR	NR	
Reference	Age	Endpoint	n	max	28	36	NR	NR	NR	NR	NR	NR	17	NR	
Cooney et al. (1989b)	0.5	BSMD MDI	274	ΔR^2	0.008	0.008	-	-	-		-	-	-	-	
Cooney et al. (1989b)	1	BSMD MDI	259	ΔR^2	0.008	0.008					-		-	-	
Cooney et al. (1989b)	2	BSMD MDI	234	ΔR^2	0.001	0.001	-	-	-	-	-	-	-	-	
Cooney et al. (1989b)	3	MSCA GCI	215	ΔR^2	0.001	0.001	-	-	-	-	-		-	-	
Cooney et al. (1989a)	4	MSCA GCI	175	adjusted R ²	-	-	0.009	0.013	0.002	0.003	0.028	-	-	-	
Cooney et al. (1991)	7	IQ (WISC-R)	175	Adjusted ß(Log BPb)	0.06		-	-0.01	-		-	-0.07	-0.06	-0.03	

Shaded cells = statistically significant association (p <0.05, 2-sided)

Shanghai, China; Yang	pu Mater	nal & Child He	alth Ce	r Pb exposure index;						
				blood Pb (µg/dL)						
				unless otherwise	Cord	Cord				
				noted	(low)	(high)	0.25 yr	0.5 yr	1 yr	
				Mean	5.3	13.4	NR	NR	NR	
				SD	1.4	2.0	NK	NK	NK	
				min	2.1	10.7	NR	NR	NR	
Reference	Age	Endpoint	n	max	7.4	17.5	NR	NR	NR	
Shen et al. (1998)	0.25	BSMD MDI	133	Adjusted MDI scores	86.8	83.4	not sig	-	-	
Shen et al. (1998)	0.5	BSMD MDI	133	Adjusted MDI scores	88.3	82	not sig	not sig	-	
Shen et al. (1998)	1	BSMD MDI	133	Adjusted MDI scores	100.3	95.1	-	not sig	not sig	
Shen et al. (1998)	1	BSMD PDI	133	Adjusted PDI scores	95.1	93.50	-	not sig	not sig	

Shaded cells = statistically significant association (p < 0.05, 2-sided)

Port Pirie, Australia				Pb exposure index;												
				blood Pb (µg/dL)			mean 0-		mean							
				unless otherwise	mean 0-5 yr	7yr	7 yr	11-13 yr	0-13 yr						Other covariates	Exclusion criteria
				Geo mean	NR	11.6	NR	7.9	14.1	-						
				SD	NR	1.4	NR	1.7	1.2	Maternal		Quality of		Subject		
				min	NR	2.1	NR	0.6	5.0	drug, alcohol		Caregiver		nutritional		
Reference	Age	Endpoint	n	max	NR	37.7	NR	30.8	31.9	& tobaco use	SES	Environment	Maternal IQ	status	delivery infert feeding method, and marital status, and subject's	
Wigg et al. (1988)	2	BSMD MDI	595	Adjusted ß							~	✓	~		family functioning, quality if the care giving environment (HOME	
McMichael et al. (1988)	4	MSCA GCI	537	Adjusted ß(Log BPb)	-				-		~	~	1		score) at 3 years, iron status, sex, age at testing, school grade,	Not reported
															ramily size, life events, birth order, birth weight, pha	
Baghurst et al. (1992)	7	IQ (WISC-R)	494	Adjusted ß(Log BPb)	-	-	-	-	-		✓	✓	~			
Tong et al.(1996)	11-13	IQ (WISC-R)	375	Adjusted &(Log BPb)	-5.1	-3.4	-4.6	-3.1	-4.3		~	~	1	~		
		Shaded cells =	= statis	tically significant assoc	ia											

Sydney, Australia				Pb exposure index;							
				blood Fb (µg/dE)							
				unless otherwise							
				noted	Maternal						
				Mean	drug,						
				SD	alcohol		Quality of		Subject		
				min	& tobaco		Caregiver		nutritional		
Reference	Age	Endpoint	n	max	use	SES	Environment	Maternal IQ	status	Other covariates	Exclusion criteria
Cooney et al. (1989b)	0.5	BSMD MDI	274	ΔR^2	✓	~	✓	✓			
Cooney et al. (1989b)	1	BSMD MDI	259	ΔR^2	✓	1	~	~		maternal age at birth, verbal IQ, education,	
Cooney et al. (1989b)	2	BSMD MDI	234	ΔR^2	✓	~	~	~		consumption during pregnancy: father's age	children of single mothers, non-English speaking mothers, mothers with drug or
Cooney et al. (1989b)	3	MSCA GCI	215	ΔR^2	✓	~	✓	~		education, and occupation; guality of the	alcohol problems, and babies with severe medical conditions, <37 weeks
Cooney et al. (1989a)	4	MSCA GCI	175	adjusted R ²		1	~	~		caregiving environment (HOME score measured	gestation, or birth weight <2,500 g
										annually) and subject gestational age, birthw	
Cooney et al. (1991)	7	IQ (WISC-R)	175	Adjusted ß(Log BPb)		✓	~	✓			

Shaded cells = statistically significant associa

Shanghai, China; Yan	ngpu Maternal & Child Hea	alth Cer Pb exposure index;						
		blood Pb (µg/dL)						
		unless otherwise						
		noted	Maternal					
		Mean	drug,				Other covariates	Exclusion criteria
		SD	alcohol		Quality of	Subject	Motomol commotional along advetaion, and at	
		min	& tobaco		Caregiver	nutritional	high abstatical complications signature and	
Reference	Age Endpoint	n max	use	SES	Environment Maternal IQ	status	birtin, obstetrical complications, cigarette and	
Shen et al. (1998)	0.25 BSMD MDI	133 Adjusted MDI scores	✓	~			alcohol and drug use during pregnancy, gravidity,	not reported
Shen et al. (1998)	0.5 BSMD MDI	133 Adjusted MDI scores					parity, premature rupture of the membranes,	
Shen et al. (1998)	1 BSMD MDI	133 Adjusted MDI scores	1	~			maternal hemoglobin during pregnancy, viral	
Shen et al. (1998)	1 BSMD PDI	133 Adjusted PDI scores	√	~			infection during pregn	

Shaded cells = statistically significant associate

Treatment of Lead Exp	osed Chi	dren (TLC) Tria	I, US	A Pb exposure index;							
				blood Pb (µg/dL) unless otherwise noted	2 yrs	5 yrs	7 yrs	peak	mean 2- 5yrs	mean 2- 7yrs	
				Mean	26.2	12.0	8.0	30.6	17.0	14.4	
				SD	5.1	5.2	4.0	6.6	5.0	4.6	
				min	NR	NR	NR	NR	NR	NR	
Reference	Age	Endpoint	n	max	NR	NR	NR	NR	NR	NR	
Chen et al. (2005)	2	BSMD MDI	780	Adjusted ß	-0.29						
Chen et al. (2005)	5	IQ (WPPSI-R	731	Adjusted ß	-0.23	-0.35			-0.29		
Chen et al. (2005)	7	IQ (WISC-III)	622	Adjusted ß	-0.11	-0.29	-0.54	-0.07		-0.33	
		Shaded cells =	statis	tically significant associ	ation (p<0	.05, 2-sideo	i)				

Rochester NY USA												6-24	6-24	6-24		
				Pb exposure index;								month	month	month		
				blood Pb (µg/dL)	lifetime	6-24			lifetime	lifetime	lifetime	average	average	average		
				unless otherwise	average	month	peak < 5	concurrent	average	average 5-	average >	< 5	5-9.9	> 10	concurrent	concurrent
				noted	< 5 yrs	average	yrs	< 5 yrs	< 5 µg/dL	9.9 µg/dL	10 µg/dL	µg/dL	µg/dL	µg/dL	< 5 µg/dL	5-9.9 µg/dL
				Mean	7.4	7.0	11.1	5.8								
				SD	4.3	3.8	7.7	4.1								
				min	NR	NR	NR	NR								
Reference	Age	Endpoint	n	max	NR	NR	NR	NR								
Canfield et al. (2003)	3	IQ (S-BIS)	171	Adjusted ß	-0.35	-0.32	-0.19	-0.31								
Canfield et al. (2003)	5	IQ (S-BIS)	171	Adjusted ß	-0.46	-0.43	-0.23	-0.46								
				n	-		-	-	64	70	40	62	79	33	107	53
Jusko et al. (2008)	6	IQ (WPPSI-R	174	Adjusted IQ	N/A	N/A	N/A	N/A	91.3	86.40	84.10	91.10	85.90	N/R	89.60	85.90
		Shaded cells =	statis	tically significant assoc	iation (p<0.	05, 2-side	d)									

				blood Pb (µg/dL)	mid											
							(mean mid					+50%				
				unless otherwise	pregnan		pregnancy					change				
				noted	CV	cord	& cord)	6 mo	1 yr	1.5 yr	2yr	0-2 yrs	2.5 yr	3 yr	3.5 yr	4 yr
				Mean	13	14.1	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
				SD	9.3	10.5	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
				min (estimated)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Reference	Age	Endpoint	n	max (estimated)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Wasserman et al. (1992)	2	Bayley MDI	392	Adjusted ß(Log BPb)	-	-3.487	-	-2.373	-3.655	-3.719	-5.307		-	-	-	-
Wasserman et al. (1994)	4	MSCA GCI	332	Adjusted ß(Log BPb)	-4.98	-7.14	•	-4.21	-7.53	-4.92	-10.44	•	-9.73	-9.35	-10.4	-9.43
				Adjusted ß												
Wasserman et al. (1997)	7	IQ (WISC-III)	309	(Log BPb AUC)	-	-	-	-	-	-	-	-	-	-	-	-
Wasserman et al. (2000)	3-7	IQ (various)	1167	Adjusted ß(Log BPb)	-	-	-6.05	-	-	-	-	-1.78	-	-	-	-
Wasserman et al. (2003)	10-12	IQ (WISC-III)	290	Adjusted ß(Log BPb)	-	-	-	-	-	-	-	-	-	-	-	-
				Adjusted												

 10-12
 IQ (WISC-III)
 167
 ß(Log tibia Pb)

 4
 Ethnicity used as a surrogate measure of socioeconomic status

 Shaded cells = statistically significant association (ρ <0.05, 2-sided)</td>
 Wasserman et al. (2003)

Treatment of Lead Exp	oosed Chi	ildren (TLC) Tria	I, USA	A Pb exposure index;							
			,	blood Pb (µg/dL) unless otherwise noted	Maternal					Other Covariates	Exclusion criteria
Reference	Age	Endpoint	n	Mean SD min max	drug, alcohol & tobaco use	SES	Quality of Caregiver Environment	Maternal IQ	Subject nutritional status	maternal IQ, parity, age at delivery, household income, prenatal smoking, race and education; and subject birth weight, gestational age at birth, iron status, HOME scale score at 24 months and	Low birth weight, preterm, English as a second language and Down syndrome
Chen et al. (2005) Chen et al. (2005) Chen et al. (2005)	2 5 7	BSMD MDI IQ (WPPSI-R IQ (WISC-III)	780 731 622	Adjusted ß Adjusted ß Adjusted ß	<i>✓</i> <i>✓</i>	* * *	✓ ✓ ✓	√ √ √	✓ ✓ ✓	 HOME Short Form (HOME-SF) at 6 years, and crowding in the home 	
		Shaded cells =	statis	tically significant associa							

Rochester, NY USA																
				Pb exposure index; blood Pb (µg/dL)	oonourrent.	nook - F	peak 5-	neek.	peak >							
				unless otherwise	concurrent	peak < 5	9.9	10 ug/dl	10						Other equations	Evolucion oritoria
				noted	> 10 µg/aL	µg/aL	µg/aL	το μα/αι	. µg/a∟						Other covariates	Exclusion chiena
				Mean						Maternal		Quality of			maternal IQ, parity, age	
				SD						drug, alcohol		Caregiver		Subject	at delivery, household	
				min						& tobacco		Environme		nutritional	income, prenatal	Low high weight protons. English op
Reference	Age	Endpoint	n	max						use	SES	nt	Maternal IQ	status	smoking, race and	Low birth weight, preterni, English as
Canfield et al. (2003)	3	IQ (S-BIS)	171	Adjusted ß						✓	~	~	1	~	education; and subject	a second language and bown
Canfield et al. (2003)	5	IQ (S-BIS)	171	Adjusted ß						✓	~	✓	~	~	birth weight, gestational	syndrome
				n	14	17	79	41	37	✓	~	✓	~	~	age at birth, iron status,	
Jusko et al. (2008)	6	IQ (WPPSI-R	174	Adjusted IQ	82.70	93.90	88.30	85.9	85.20	✓	~	✓	√	✓	HOME scale score at 24	
		Shaded cells =	statis	tically significant associa	a.											

Yugoslavia Prospective Lead Study				Pb exposure index; blood Pb (µg/dL) unless otherwise	BPb AUC (BPb - AUC 0-7	+50% change	mean 0-	10-13 vr								
				noted	5 yrs	vrs	2-7 yrs	10 yrs	10 yr	tibia Pb?						Other covariates	Exclusion criteria
				Mean	NR	NR	NR	NR	NR		Maternal						
				SD	NR	NR	NR	NR	NR	NR	drug,		Quality of				
- /				min (estimated)	NR	NR	NR	NR	NR	NR	alcohol &		Caregiver	Maternal	Subject nutritional		
Reference	Age	Endpoint	n	max (estimated)	NR	NR	NR	NR	NR	NR	tobaco use	SES	Environment	IQ	status		destation <28 weeks or >44 weeks
Wasserman et al. (1992)	2	Bayley MDI	392	Adjusted ß(Log BPb)	-			-	-	-						Subject ethnicity (surrogate for SES).	multiple births, residence more than
Wasserman et al. (1994)	4	MSCA GCI	332	Adjusted ß(Log BPb)	-			-	-	-						birthweight, age at assessment, gender,	10 km from the community pediatric
				Adjusted ß												sibship size, haemoglobin, and maternal	center, and those with chromosomal
Wasserman et al. (1997)	7	IQ (WISC-III)	309	(Log BPb AUC)	sig	-0.2146		-	-	-		\checkmark^4	~	~	~	IQ, education, parity, age,	abnormalities or nervous system
Wasserman et al. (2000)	3-7	IQ (various)	1167	Adjusted ß(Log BPb)	-	-	-2.71	-	-	-		\checkmark^4	~	~	~		defects
Wasserman et al. (2003)	10-12	IQ (WISC-III)	290	Adjusted ß(Log BPb)	-	-	-	-5.02	-4.48			\checkmark^4	✓	~	✓	_	
Wasserman et al. (2003)	10-12	IQ (WISC-III)	167	Adjusted ß(Log tibia Pb)	-	-	-	-	-	-11.06		\checkmark^4	~	~	~	_	

4 Ethnicity used as a surrogate measure of soci Shaded cells = statistically significant associa