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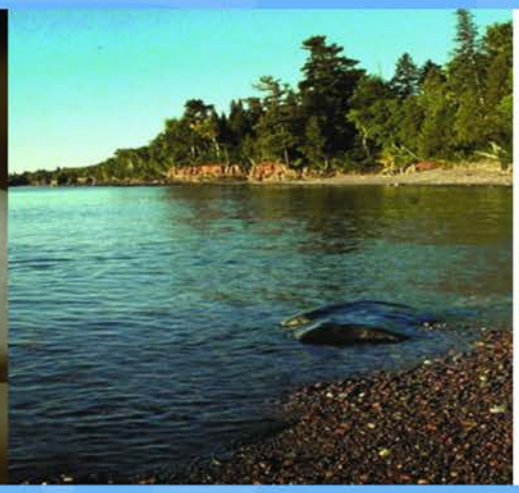
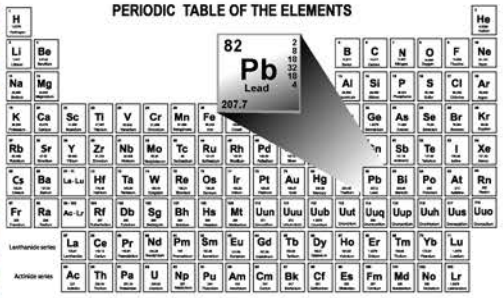
Agency

Integrated Science Assessment for Lead





Contains Errata Sheet created 5/12/2014

Office of Research and Development
National Center for Environmental Assessment, Research Triangle Park, NC

United States Environmental Protection Agency

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Integrated Science Assessment for Lead

National Center for Environmental Assessment-RTP Division
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, NC

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For the document titled: Integrated Science Assessment (ISA) for Lead(Pb), June 2013, Final

Table or Figure	Page	Correction or Comments
	3-37	Replaced "at a concentration of 0.001 µg/m^3" with "at a concentration of 1 µg/m^3"
Figure 4-2, Table 4-3, Figure 4-15, Table 4-16	4-62, 4-70, 4-72, 4-76, 4-124, 4-254, 4-256, 4-284, 4-285	Two errors were identified in the dataset analyzed by Lanphear et al. (2005) that resulted in slight modifications to values reported in the ISA. The page numbers of the ISA that are affected by these errors are noted here; however, the final ISA has not been corrected. Rather, recalculations using the corrected dataset are presented in the Table on page A-2 of the memo to the docket (Kirrane and Patel, 2014), EPA-HQ-ORD-2011-0051-0050. EPA has concluded that the conclusions drawn in the 2013 Pb ISA are not materially affected by these newly identified errors.
Table 6-5, column 5	6-307	Replaced "delayed in the 100 mg/L treatment" with "delayed in the 100 µg Pb/L treatment"

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Acronyms and Abbreviations

Acronym/Abbreviation	Meaning
α	alpha
αT	the extent of DNA denaturation per cell
Å	Ångström (10^{-10} meter)
AA	African American; arachidonic acid, atomic absorption
AALM	All Ages Lead Model
AAS	atomic absorption (spectrophotometry, spectrometry, spectroscopy)
Ab	amyloid-beta peptide
ABL	atmospheric boundary layer
ACE	angiotensin converting enzyme
ACF	Apalachicola, Chattahoochee, and Flint River Basin
ACh	acetylcholine
ACP	acid phosphatase
ACR	acute to chronic ratio
Acyl-CoA	acyl-coenzyme A
AD	axial diffusivity
ADHD	attention deficit hyperactivity disorder
ADP	adenosine diphosphate
AE	anion exchanger
AEC	adenylate energy charge
AERMOD	atmospheric dispersion model
AF	absorbed fraction; absorption fraction
aff'd	affirmed
A/G	albumin/globulin
Ag	silver
AGL	above-ground level
A-horizon	Topsoil horizon (surface soil)
AKI	acute kidney injury
Al	aluminum
ALA	aminolevulinic acid
ALAD	aminolevulinic acid dehydratase;

Acronym/Abbreviation	Meaning
ALAD 1-1	aminolevulinate delta-dehydratase 1-1
ALAD-2	aminolevulinate delta-dehydratase-2
ALD	aldehyde dehydrogenase
ALM	Adult Lead Methodology
ALP	alkaline phosphatase
ALS	Amyotrophic Lateral Sclerosis (Lou Gehrig's disease)
ALT	alanine aminotransferase
AM	Alveolar macrophages
AMF	arbuscular mycorrhizal fungi
AMP	adenosine monophosphate
ANC	acid neutralizing capacity; absolute neutrophil counts
ANF	atrial natriuretic factor
AngII	renal angiotensin II
ANOVA	analysis of variance
ANPR	advance notice of proposed rulemaking
AOP	adverse outcome pathway
AP-1	activator protein-1
ApaI	polymorphism of the VDR in humans
APC	antigen-presenting cell
APOE	Apolipoprotein E
APRT	adenine phosphoribosyltransferase
AQCD	Air Quality Criteria Document
AQS	(U.S. EPA) Air Quality System (database)
Ar	argon
As	arsenic
AST	aspartate aminotransferase
ASV	anode stripping voltammetry
ATLD	ataxia-telangiectasia-like disorder

Acronym/Abbreviation	Meaning
ATOFMS	aerosol time-of-flight mass spectrometry
ATP	adenosine-triphosphate
ATPase	adenosine triphosphatase; adenosine triphosphate synthase
ATS	American Thoracic Society
ATSDR	Agency for Toxic Substances and Disease Research
Au	gold
avg	average
AVS	acid-volatile sulfides
a-wave	initial negative deflection in the electroretinogram
AWQC	Ambient Water Quality Criteria
β	Beta; Beta coefficient; regression coefficient; standardized coefficient
3β-HSD	3-beta-hydroxysteroid dehydrogenase
17β-HSD	17-beta-hydroxysteroid dehydrogenase
Ba	barium
BAF	bioaccumulation factors
BAL	2,3-dimercaptopropanol
BASC	Behavior Assessment System for Children
BASC-PRS	Behavior Assessment System for Children-Parent Ratings Scale
BASC-TRS	Behavior Assessment System for Children-Teacher Rating Scale
BC	black carbon, soot
BCB	blood cerebrospinal fluid barrier
B-cell	Bone marrow-derived lymphocytes, B lymphocyte
BCF	bioconcentration factors
Bcl-x	member of the B-cell lymphoma-2 protein family
Bcl-xl	B-cell lymphoma-extra large
B-horizon	subsoil horizon
bio	biological
Bi_2S_3	bismuth (III) sulfide
BK	biokinetics
BLM	biotic ligand model
BMD	benchmark dose; bone mineral density
BMDL	benchmark dose limit
BMI	body mass index
BMP	bone morphogenetic protein
BMS	Baltimore Memory Study
BMW	battery manufacturing workers
BP	blood pressure
Br	bromine
BR	bronchial responsiveness
BrdU	bromo-2'-deoxyuridine
8-Br-GMPc	8-bromo-cyclic guanosine monophosphate
Bs-horizon	subsoil horizon with accumulation of sesquioxides
BSI	Brief Symptom Inventory
BSID-II	Bayley Scale for Infant Development-II
BsmI	polymorphism of the VDR in humans
Bt20	Birth-to-age Twenty (cohort)
BUN	blood urea nitrogen
bw	body weight
b-wave	initial positive deflection in the electroretinogram
C	carbon; Celsius; soil or dry sediment Pb concentration; Caucasian; Cysteine
Ca	calcium
Ca^{2+}	calcium ion
CAA	Clean Air Act
CaBP	calcium binding protein
$CaCl_2$	calcium chloride
$CaCO_3$	calcium carbonate; calcite
CaEDTA	calcium ethylenediaminetetraacetic acid
CaMKII	calmodulin-dependent protein kinase II

Acronym/Abbreviation	Meaning
cAMP	cyclic adenosine monophosphate
CASAC	Clean Air Scientific Advisory Committee
CASM	Comprehensive Aquatic Systems Model
$CaSO_4$	calcium sulfate
$CaSO_4 \cdot 2H_2O$	gypsum
CAT	catalase
CBLI	cumulative blood Pb index
CBSA	core based statistical area
CCSEM	computer-controlled scanning electron microscopy
CD	cluster of differentiation
Cd	cadmium
Cd(II)	cadmium (II)
Cd^{2+}	cadmium ion
CD3+	T lymphocyte
CD4+	T helper cell
CDC	Centers for Disease Control
CEA	carcinoembryonic antigen
CEC	cation exchange capacity
cent	central
cert.	certiorari
cf	correction factor; latin abbreviation for conferre (used as "compared with)
CFL	constant flux layer
CFR	Code of Federal Regulations
cGMP	cyclic guanosine monophosphate
C-H	carbon-hydrogen (bond)
CHAD	Consolidated Human Activity Database
ChAT	chlorine acetyltransferase
CHD	coronary heart disease
CHL	Chinese hamster lung
CHO	Chinese hamster ovary cell line
C-horizon	Soil horizon underneath A- and B-horizons, may contain lumps or shelves of rock and parent material

Acronym/Abbreviation	Meaning
CHV79	Chinese hamster lung cell line
CI	confidence interval
Cir.	circuit
CKD	chronic kidney disease
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CL	confidence limit
Cl	chlorine
Cl^-	chlorine ion
Cl_2	molecular chlorine
CLACE 5	Fifth Cloud and Aerosol Characterization Experiment in the Free Troposphere campaign
CLS	Cincinnati Lead Study
CO	carbon monoxide
CO_2	carbon dioxide
CO_3^{2-}	carbonate ion
Co	cobalt
CoA	coenzyme A
COD	coefficient of difference
Coeff	coefficient
COMP aT	The percentage of sperm with increased sensitivity to DNA denaturation
Con	control
Conc.	concentration
Cong.	congress
Corr	correlation
COX	cyclooxygenase; cytochrome oxidase subunits
COX-2	cyclooxygenase-2
$cPLA_2$	cytosolic phospholipidase A_2
CPRI	coarse particle rotary impactor
CPRS-R	Conners' Parent Rating Scale-Revised
Cr	chromium; creatine
C-R	concentration-response (relaltionship)
Cr III	chromium III
CRAC	Ca^{2+} release activated calcium

Acronym/Abbreviation	Meaning
CRACI	calcium release activated calcium influx
CREB	cyclic adenosinemonophosphate (cAMP) response element-binding
CRP	C-reactive protein
CSF	colony-stimulating factor
CSN	Chemical Speciation Network
CT	zinc-adequate control
Cu	copper
Cu(II)	copper (II)
CV	coefficient of variation
CVD	cardiovascular disease
CYP	cytochrome
CYP 1A1, Cyp1A1	cytochrome P450 family 1 member A1
CYP 1A2, Cyp1A2	cytochrome P450 family 1 member A2
CYP P450	cytochrome P450
Δ	delta, difference, change
Δ5-3β-HSD	delta-5-3-beta-hydroxysteroid dehydrogenase
δ-ALA	5-aminolevulinic acid; delta-aminolevulinic acid
δ-ALAD	delta-aminolevulinic acid dehydratase
D_2, D_3	dopamine receptors
D50	size at 50% efficiency
d	day(s); depth
db, dB	decibel
DbH	dopamine beta-hydroxylase
DBP	diastolic blood pressure
DENA	Denali National Park and Preserve, Alaska
dep	dependent
dev.	deviation
DEX	exogenous dexamethasone
DG	degenerate gyrus
2-dG	2-deoxyguanosine
DHAA	dehydroascorbate
diff	differentiation
DIT	developmental immunotoxicity
DMPS	2,3-dimercaptopropane-l-sulfonic acid
DMSA	dimercaptosuccinic acid
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DoAD	developmental origins of adult disease
DOC	dissolved organic carbon
DOM	dissolved organic matter
DP-109	metal chelator
DP-460	metal chelator
DR	diet-restricted
DRD4	dopamine 4 receptor
DRD4.7	dopamine 4 receptor repeat alleles
DRUM	Davis Rotating Unit for Monitoring
D-serine	neuronal signal
DSM-IV	Diagnostic Statistical Manual-IV
DTH	delayed-type hypersensitivity
DTPA	diethylene triamine pentaacetic acid; technetium-diethylenetriamine-pentaacetic acid
E	east; expression for exposure
E2	estradiol
e	exponential function
EC	elemental carbon, endothelial cell
EC_{10}	effect concentration for 10% of test population
EC_{20}	effect concentration for 20% of test population
EC_{50}	effect concentration for 50% of test population
ECG	electrocardiography; electrocardiogram
ECOD	7-ethoxycoumarin-o-deethylase
Eco-SSLs	ecological soil screening levels
ED_{10}	effect dose for 10% of population

Acronym/Abbreviation	Meaning
EDTA	ethylenediaminetetraacetic acid
EFS	electrical field stimulus
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
eGFR	estimated glomerular filtration rate
Eh	electrochemical potential
E-horizon	Eluviated horizon; soil horizon which is eluviated or leached of mineral and/or organic content
EI-MS	electron impact ionization mass spectrometry
ELPI	electrical low-pressure impactor
eNOS	endothelial nitric oxide synthase
EOG	end-of-grade
EPA	U.S. Environmental Protection Agency
EPT	ephemeroptera, plecoptera, trichoptera
ER	endoplasmic reticulum
Erg-1	ether-a-go-go related gene
ERG	electroretinogram
ERK	extracellular signal regulated kinase
ERK1/2	extracellular signal-regulated kinases 1 and 2
EROD	7-ethoxyresorufin-o-deethylase
ESCA	electron spectroscopy for chemical analysis
ESI-MS	electrospray ionization mass spectrometry
ESRD	end stage renal disease
ET	endothelin
ET-1	vasoconstrictor endothelin-1
ET_A-type receptors	endothelin type A receptors
ETS	environmental tobacco smoke
EU	European Union
EURO	European emission standard
eV	electronvolts
EXAFS	X-ray absorption fine structure spectroscopy
F_0	filial “zero” generation (parental stock)
F_1	first filial generation (offspring of F_0)
F_2	second filial generation (offspring of F_1)
FAA	Federal Aviation Agency
FAI	free androgen index
FAS	apoptosis stimulating fragment
Fas-L	apoptosis stimulating fragment ligand
Fe	iron
Fe(III)	iron III
FEM	Federal equivalence method
FEV1	forced expiratory volume in 1 second
FI	fixed interval
FI-Ext	fixed interval with extinction
Fl	fluoride
FokI	polymorphism of the VDR in humans
FR	Federal Register (Notice)
FrA	fractional anisotropy
FR-FI	fixed ratio-fixed interval
FRM	Federal reference method
FSH	follicle-stimulating hormone
FSIQ	full scale intelligence quotient (IQ)
FT3	free triiodothyronine
FT4	free thyroxine
G	pregnancy; guanine
G2	gap 2 Phase
g, kg, mg, µg, ng, pg	gram(s), kilogram(s), milligram(s), microgram(s), nanogram(s), picogram(s)
G93A	mouse model
GAAR	Gates of the Arctic National Park and Preserve, Alaska
GABA	γ-aminobutyric acid; gamma aminobutyric acid

Acronym/Abbreviation	Meaning
GABAergic	inhibitory neurons that release the neurotransmitter GABA
GAD	generalized anxiety disorder
GC	gas chromatography
G-CSF	granulocyte colony-stimulating factor
GD	gestational day
GEE	generalized estimating equations
GFAAS	graphite furnace atomic absorption spectrometry
GFAP	glial fibrillary acidic protein
GFR	glomerular filtration rate
GGT	gamma-glutamyl transpeptidase
GH	growth hormone
GHRH	growth-hormone releasing hormone
GI	gastrointestinal
GIS	Geographic Information System
G+L	pregnancy plus lactation
GLAC	Glacier National Park, Montana
GLE	gestationally-lead exposed
GM	geometric mean
GMR	geometric mean blood Pb ratio
GnRH	gonadotropin-releasing hormone
G6PD	glucose-6-phosphate dehydrogenase
GPEI	glutathione transferase P (GST-P) enhancer I
GPT	glutamate pyruvate transaminase
GPx	glutathione peroxidase
GPX1	gene encoding for glutathione peroxidase 1
GR	glutathione reductase
GRP78	glucose-regulated protein 78
GRP94	glucose-regulated protein 94
Grp	glucose-regulated protein
GSD	geometric standard deviation
GSH	glutathione
GSSG	glutathione disulfide
GST	glutathione S-transferase
GSTM1	glutathione S-transferase Mu 1
GST-P	glutathione transferase P
GTP	guanosine-5'-triphosphate; guanine triphosphate
H	hydrogen
H^+	hydrogen ion
h	hour(s)
ha	hectare
HAD	hydroxy-alkenals
HAP	hazardous air pollutant
Hb	hemoglobin
HC_5	acute toxicity hazardous concentration for 5% of species
HC_{10}	acute toxicity hazardous concentration for 10% of species
HCl	hydrochloric acid
HCO_3^-	bicarbonate; hydrogen carbonate
Hct	hematocrit
HDL	high-density lipoprotein
HERO	Health and Environmental Research online (database)
HEW	U.S. Department of Health, Education, and Welfare
HF	hydrogen fluoride
HFE	hemochromatosis gene
HFE C282Y	hemochromatosis gene with C282Y mutation
HFE H63D	hemochromatosis gene with H63D mutation
Hg	mercury
$HgCl_2$	mercury(II) chloride
5-HIAA	5-hydroxyindoleacetic acid
HIV	human immunodeficiency virus
HLA-DRB	human leukocyte antigen genes
HMEC	human dermal microvascular endothelial cells

Acronym/Abbreviation	Meaning
HMGR	3-hydroxy-3-methylglutaryl-CoA reductase
HMOX-1	heme oxygenase-1
HNO_3	nitric acid
HO-1	heme oxygenase; heme oxidase-1
H_2O	water
H_2O_2	hydrogen peroxide
HOME	Home Observation for Measurement of the Environment
HPA	hypothalamic-pituitary-adrenal
HPb, h-Pb	high Pb
HPG	hypothalamic-pituitary-gonadal
HPLC	high-performance liquid chromatography
HPRT	hypoxanthine-guanine phosphoribosyltransferase
HPT	hyperparathyroidism; hypothalamic-pituitary-thyroid
HR	heart rate; hazard ratio
HRV	heart rate variability
hsp	heat shock proteins
5HT	serotonin
5-HT	5-hydroxytryptamine
5-HT2B	5-hydroxytryptamine receptor 2B
hTERT	telomerase reverse transcriptase
HVA	homovanillic acid
I	interstate
IARC	International Agency for Research on Cancer
IC_{50}	half maximal inhibitory concentration
ICAP	inductively coupled argon plasma
ICP-AES	Inductively coupled plasma atomic emission spectroscopy
ICPMS, ICP-MS	Inductively coupled plasma mass spectrometry
ICR	imprinting control region
ICRP	International Commission on Radiological Protection
ID	identification
IDA	iron-deficiency anemia
IDE	insulin-degrading enzyme
IEPA	Illinois Environmental Protection Agency
IEUBK	Integrated Exposure Uptake Biokinetic
IFN-γ	interferon-gamma
Ig	immunoglobulin
IgA	immunoglobulin A
IgE	immunoglobulin E
IGF-1	insulin-like growth factor 1
IgG	immunoglobulin G
IgM	immunoglobulin M
IHD	ischemic heart disease
IL	interleukin
IL-1β	interleukin-1 Beta
IL-2	interleukin-2
IL-4	interleukin-4
IL-5	interleukin-5
IL-6	interleukin-6
IL-8	interleukin-8
IL-10	interleukin-10
IL-12	interleukin-12
IMPROVE	Interagency Monitoring of Protected Visual Environment
IMT	intimal medial thickening
INL	inner neuroblastic layers of the retina
iNOS	inducible nitric oxide synthase
IOM	Institute of Medicine (provides health information to the NAS [National Academy of Sciences])
i.p.	intraperitoneal (route)
IQ	intelligence quotient
IQR	interquartile range
IRE1	inositol-requiring enzyme-1
IRP	integrated review plan
ISA	Integrated Science Assessment

Acronym/Abbreviation	Meaning
ISC-PRIME	Industrial Source Complex-Plume Rise Model Enhancements
ISF	intake slope factor
ISL	inertial sublayer
ISO	International Standards Organization
i.v.	intravenous
IVBA	in vitro bioaccessibility
IVF	in vitro fertilization
JNK	jun N-terminal kinase
K	Kelvin (temperature); potassium; resuspension factor
K^+	potassium ion
$K_{0.5}$	concentration of free metal giving half maximal metal-dependent release
KART	Karters of American Racing Triad
K_d	dissociation constant
Kd	partition coefficient; ratio of the metal concentration in soil to that in soil solution
kDa, kD	kiloDalton
KEDI-WISC	Korean Educational Development Institute-Wechsler Intelligence Scale for Children
6-keto-PGF1α	6-keto-prostaglandin F1α (vasodilatory prostaglandin)
keV	kiloelectron volt
Ki-67	antigen, cell cycle and tumor growth marker
Kim-1	kidney injury molecule-1
Kinder-KITAP	Kinder-Testbatterie zur Aufmerksamkeitsprüfung für Kinder
K-ras	specific proto-oncogene
K-XRF	K-x-ray fluorescence method of scanning for bone Pb
Λ	lambda; resuspension rate
L	length
L, dL, mL	Liter(s) [1000 mL/L], deciliter(s) [100 mL/dL], milliliter(s) [1 mL/mL]
LA-ICP-MS	laser ablation inductively coupled plasma mass spectrometry
LC_{50}	lethal concentration (at which 50% of exposed organisms die)
LD_{50}	lethal dose (at which 50% of exposed organisms die)
LDH	lactate dehydrogenase
LDL	low-density lipoproteins
LFH-horizons	organic soil horizons located above well-drained surface soil
LF/HF	low frequency to high frequency ratio
LH	luteinizing hormone
LHRH	luteinizing hormone releasing hormone
LINE	long interspersed nuclear element
LINE-1	long interspersed nucleotide elements-1
LLNA	local lymph node assay
ln	natural logarithm
L-NAME	L-NG-nitroarginine methyl ester
L-NOARG	L-nitroarginine
LOD	limit of detection
LOEC	lowest-observed-effect concentration
log	logarithm
LPb	low Pb
LPS	lipopolysaccharide
LSO	lateral superior olive
LTP	long-term potentiation
M	metal
M, mM, μM, nM, pM	Molar, millimolar (10^{-3} M), micromolar (10^{-6} M), nanomolar (10^{-9} M), picomolar (10^{-12} M)
m, km, cm, mm, μm, nm	meter(s), kilometer(s), centimeter(s), millimeter(s), micrometer(s) [micron(s)], nanometer(s)
MAP	mean arterial pressure
MAPK	mitogen-activated protein kinase(s), MAP kinase

Acronym/Abbreviation	Meaning
MATC	maximum acceptable toxicant concentration
max	maximum, maxima
MBP	myelin basic protein
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MchDMSA	mono-cyclohexyl dimercaptosuccinic acid
MCL	maximum containment level
MCP-1	monocyte chemotactic protein-1
MCV	mean corpuscular volume
MD	mean diffusivity
MDA	malondialdehyde
MDD	major depressive disorder
MDI	Mental Development Index
MDL	method detection limit
MDRD	Modification of Diet in Kidney Disease
Med, med	median
MEK1	dual specificity mitogen-activated protein kinase 1
MEK2	dual specificity mitogen-activated protein kinase 2
MENTOR	Modeling Environment for Total Risk (framework)
Mg	magnesium
Mg^{2+}	magnesium ion
MHC	major histocompatibility complex
MI	myocardial infarction, "heart attack;" myocardial ischemia
mI	myoinositol
min	minimum; minima; minute(s)
MKK1/2	MAPK kinase 1 and 2
ML	mixed layer
MMAD	mass median aerodynamic diameter
MMDD	mental retardation or developmental disabilities
MMF	mycophenolate mofetil
mmHg	millimeters of mercury
mmol, μmol, nmol	millimole(s), micromole(s), nanomole(s)
MN	micronuclei formation; mononuclear
Mn	manganese
MNE	micronucleated erythrocytes per thousand
MnO_2	manganese dioxide
Mo	molybdenum
mo	month(s)
MOA(s)	mode(s) of action
MORA	Mount Rainier National Park, Washington State
MOUDI	multi-orifice uniform deposit impactor
MPb, m-Pb	moderate Pb
MPO	myeloperoxidase
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
MRS	magnetic resonance spectroscopy
MS	maternal stress
MSC	mesenchymal cell
MSWI	municipal solid waste incineration
Mt	metallothionein
MTHFR	methylenetetrahydrofolate reductase
MTP	mitochondrial transmembrane pore
MW	molecular weight
MZ	marginal zinc
N	nitrogen; normal; north; number; population
n	number of observations
Na	sodium
Na^+	sodium ion
NAAQS	National Ambient Air Quality Standards
NAC	N-acetyl cysteine; nucleus accumbens
$Na_2CaEDTA$	calcium disodium ethylenediaminetetraacetic acid

Acronym/Abbreviation	Meaning
NaCl	sodium chloride
NAD	nicotinamide adenine dinucleotide
NADH	nicotinamide adenine dinucleotide dehydrogenase
NADP	nicotinamide adenine dinucleotide phosphate
NADPH, NAD(P)H	reduced nicotinamide adenine dinucleotide phosphate
NAEC	no-adverse-effect concentration
NAG	N-acetyl-β-D-glucosaminidase; N-acetylglucosamine
$NaHCO_3$	sodium bicarbonate; sodium hydrogen carbonate
NANC	non-adrenergic non-cholinergic
NAS	U.S. Department of Veteran's Affair's Normative Aging Study; National Academy of Sciences
NASCAR	National Association for Stock Car Automobile Racing
NATTS	National Air Toxics Trends Station
NAWQA	National Water Quality Assessment
NCAM	neural cell adhesion molecule
NCEA	National Center for Environmental Assessment
NCore	National Core multi-pollutant monitoring network
N.D.	not detected
NDMAR	N-nitrosodimethylamine receptor
NE	norepinephrine
NECAT	New England Children's Amalgam Trial
NEI	National Emissions Inventory
NFI	non-fixed interval
NF-κB	nuclear factor kappa B
NGAL	neutrophil gelatinase-associated lipocalin
NGF	nerve growth factor
NH	non-hispanic
NHANES	National Health and Nutrition Examination Survey
NH_4Cl	ammonium chloride
NHEJ	non-homologous end joining
NHEXAS	National Human Exposure Assessment Survey
NH_4OAc	ammonium acetate
7-NI	7-nitroinidazole
Ni	nickel
NICA	non-ideal competitive absorption
NIOSH	National Institute for Occupational Safety and Health
NIST	National Institute of Standards and Technology
NK	natural killer
NKF-K/DOQI	National Kidney Foundation - Kidney Disease Outcomes Quality Initiative
NMDA	N-methyl-D-aspartate
NMR	nuclear magnetic resonance
nNOS	neuronal nitric oxide synthase (NOS)
NO	nitric oxide; nitric oxide radical, nitrogen monoxide
NO_2	nitrogen dioxide
No.	number
NOAA	National Oceanic and Atmospheric Administration
NOAEL	no observed adverse effect level
NOAT	Noatak National Preserve, Alaska
NOCA	North Cascades National Park, Washington State
NOEC	no-observed-effect concentration
NOEL	no-observed-effect level
NOS	nitric oxide synthase; nitric oxide systems
NO_X	nitrogen oxides, oxides of nitrogen ($NO + NO_2$)
NP	nanoparticle
NPSH	nonprotein sulfhydryl
NQO1	NAD(P)H-quinone oxidoreductase (genotype)

Acronym/Abbreviation	Meaning
NRC	National Research Council
NRCS	Natural Resources Conservation Service
Nrf2	nuclear factor erythroid 2-related factor 2
NS	not specified
NTP	National Toxicology Program
NTPDase	nucleoside triphosphate diphosphohydrolase
NW	northwest
NYC	New York City
NZ	New Zealand
O_2	molecular oxygen
O_2^-	superoxide, superoxide free radical
O_3	ozone
9-O-Ac-GD3	9-O-acetylated-GD3
OAQPS	U.S. EPA Office of Air Quality Planning and Standards, in OAR
OAR	U.S. EPA Office of Air and Radiation
OBS	observations
OC	organic carbon
OEPA	Ohio Environmental Protection Agency
OH^-	hydroxide ion
1,25-$(OH)_2$D3	1,25-dihydroxy vitamin D
O-horizon	horizon forest floor, organic soil horizon (above surface soil)
OLC	osteoblast-like cells
OLYM	Olympic National Park, Washington State
OM	organic matter
ONL	outer neuroblastic layers of the retina
$ONOO^-$	peroxynitrate ion
OR	odds ratio
ORD	U.S. EPA Office of Research and Development
OS	offspring stress
OSHA	Occupational Safety and Health Administration
OVA	ovalbumin
8-oxo-dG	8-hydroxy-2'-deoxyguanosine
P	percentile; phosphorus
P_0	parent generation
P450	cytochrome P450
p	probability value; number of paired hourly observations; statistical significance
PA	policy assessment
PAD	peripheral arterial disease
PAH(s)	polycyclic aromatic hydrocarbon(s)
Pb	lead
^{203}Pb	lead-203 radionuclide
^{204}Pb	stable isotope of lead-204
^{206}Pb	stable isotope of lead-206
^{207}Pb	stable isotope of lead-207
^{208}Pb	stable isotope of lead-208
^{210}Pb	stable isotope of lead-210
Pb^{++}	divalent Pb ion
Pb^0	elemental lead
Pb(II)	lead (II)
Pb^{2+}	lead ion
$Pb(Ac)_2$	lead acetate
PbB	blood lead concentration
PbBrCl	lead bromochloride
$Pb(C_2H_3O_2)_2$	lead (II) acetate
$PbCl^+$	lead chloride
$PbCl_2$	lead chloride
$PbCl_3$	lead (III) chloride; lead trichloride
$PbCl_4$	lead (IV) chloride; lead tetrachloride
$PbCO_3$	cerussite; lead carbonate
$Pb(CO_3)_2$	lead (IV) carbonate
$Pb(CO_3)_2(OH)_2$	hydrocerussite
$PbCrO_4$	lead (II) chromate
PbD	floor dust lead

Acronym/Abbreviation	Meaning
$PbFe_6(SO_4)_4(OH)_{12}$	plumbjarosite
PBG	porphobilinogen
$Pb(NO_3)_2$	lead(II) nitrate
Pb-NS	lead-no stress
PbO	lead oxide; litharge; massicot
PbO_2	lead dioxide
$Pb(IV)O_2$	lead dioxide
Pb_3O_4	minimum or "red Pb"
$Pb(OH)_2$	lead hydroxide
$Pb_5(PO_4)_3Cl$	pyromorphite
$Pb_5(PO_4)_3OH$	hydroxypyromorphite
PbS	galena; lead sulfide; soil lead concentration
PbSe	lead selenide
$PbSO_4$	anglesite; lead sulfate
$Pb_4SO_4(CO_3)_2(OH)_3$	macphersonite
PbxS	lead by stress
$Pb_5(VO_4)_3Cl$	vanadinite
PC12	pheochromocytoma 12 (adrenal / neuronal cell line)
PCA	principal component analysis
PCE	polychromatic erythrocyte
PCR	polymerase chain reaction
Pct	percent
PCV	packed cell volume
PD	Parkinson's disease
PDI	Psychomotor Development Index
PEC	probable effect concentration
PEL	permissible exposure limit
PER	partial exfiltration reactor
PG	prostaglandin
PGE_2, PGE2	prostaglandin E_2
PGF_2	prostaglandin F2
pH	relative acidity; Log of the reciprocal of the hydrogen ion concentration
PHA	polyhydroxyalkanoates
PHE	phenylalanine

Acronym/Abbreviation	Meaning
PIH	pregnancy induced hypertension
PIQ	performance intelligence quotient (IQ)
PIR	poverty-income ratio
PIXE	particle induced X-Ray emission; proton-induced x-ray emission
PKC	protein kinase C
PLP	proteolipid protein
PM	particulate matter
PM_X	Particulate matter of a specific size range not defined for regulatory use. Usually X refers to the 50% cut point, the aerodynamic diameter at which the sampler collects 50% of the particles and rejects 50% of the particles. The collection efficiency, given by a penetration curve, increases for particles with smaller diameters and decreases for particles with larger diameters. The definition of PMX is sometimes abbreviated as “particles with a nominal aerodynamic diameter less than or equal to X μm” although X is usually a 50% cut point.

Acronym/Abbreviation	Meaning
PM_{10}	In general terms, particulate matter with an aerodynamic diameter less than or equal to a nominal 10 μm; a measurement of thoracic particles (i.e., that subset of inhalable particles thought small enough to penetrate beyond the larynx into the thoracic region of the respiratory tract). In regulatory terms, particles with an upper 50% cut-point of 10 ± 0.5 μm aerodynamic diameter (the 50% cut point diameter is the diameter at which the sampler collects 50% of the particles and rejects 50% of the particles) and a penetration curve as measured by a reference method based on Appendix J of 40 CFR Part 50 and designated in accordance with 40 CFR Part 53 or by an equivalent method designated in accordance with 40 CFR Part 53.
$PM_{2.5}$	In general terms, particulate matter with an aerodynamic diameter less than or equal to a nominal 2.5 μm; a measurement of fine particles. In regulatory terms, particles with an upper 50% cut-point of 2.5 μm aerodynamic diameter (the 50% cut point diameter is the diameter at which the sampler collects 50% of the particles and rejects 50% of the particles) and a penetration curve as measured by a reference method based on Appendix L of 40 CFR Part 50 and designated in accordance with 40 CFR Part 53, by an equivalent method designated in accordance with 40 CFR Part 53, or by an approved regional method designated in accordance with Appendix C of 40 CFR Part 58.
$PM_{10-2.5}$	In general terms, particulate matter with an aerodynamic diameter less than or equal to a nominal 10 μm and greater than a nominal 2.5 μm; a measurement of thoracic coarse particulate matter or the coarse fraction of PM10. In regulatory terms, particles with an upper 50% cut-point of 10 μm aerodynamic diameter and a lower 50% cut-point of 2.5 μm aerodynamic diameter (the 50% cut point diameter is the diameter at which the sampler collects 50% of the particles and rejects 50% of the particles) as measured by a reference method based on Appendix O of 40 CFR Part 50 and designated in accordance with 40 CFR Part 53 or by an equivalent method designated in accordance with 40 CFR Part 53.
PM_{10C}	The PM10-2.5 concentration of PM10-2.5 measured by the 40 CFR Part 50 Appendix O reference method which consists of currently operated, co-located low-volume (16.7 Lpm) PM10 and PM2.5 reference method samplers.
p38MAPK	p38 mitogen-activated protein kinase(s)
PMN	polymorphonuclear leukocyte
P5N	pyrimidine 5'-nucleotidase
PND	post natal day
POC	particulate organic carbon
PP	polypropylene; pulse pressure
ppb	parts per billion
ppm	parts per million
PRP	post-reinforcement pause
PS	dam stress; prenatal stress; phosphatidylserine
PSA	prostate specific antigen
PSA-NCAM	polysialylated-neural cell adhesion molecule
PT	proximal tubule
PTFE	polytetrafluoroethylene
PTH	parathyroid hormone

Acronym/Abbreviation	Meaning
PTHrP	parathyroid hormone-related protein
PUFA	polyunsaturated fatty acid
PVC	polyvinyl chloride
PVD	peripheral vascular disease
Q	quantile; quartile; quintile
QRS	QRS complex in ECG
QT	QT interval in ECG
QTc	corrected QT Interval
ρ	rho; bulk density; correlation
ρS	Pearson's r correlation coefficient
R	net drainage loss out of soil depth of concern; Spearman correlation coefficient; upward resuspension flux; correlation
r	Pearson correlation coefficient
R^2	multiple regression correlation coefficient
r^2	correlation coefficient
RAAS	renin-angiotensin-aldosterone system
RAC2	gene encoding for Rac2
RBA	relative bioavailability
RBC	red blood cell
RBP	retinol binding protein
RD	radial diffusivity
REA	Risk/Exposure Assessment
Ref	reference (group)
RI-RI	concurrent random interval
RL	repeated learning
^{220}Rn	radon isotope
^{222}Rn	stable isotope of radon-222
RNA	ribonucleic acid
ROI	reactive oxygen intermediate/superoxide anion; regions of interest
ROMO	Rocky Mountain National Park, Colorado
ROS	reactive oxygen species
RR	relative risk; risk ratio
RSL	roughness sublayer (transition layer, wake layer, interfacial layer)
rtPCR	reverse transcription polymerase chain reaction
σ	sigma, standard deviation
S	south; sulfur; synthesis phase
SAB	U.S. EPA Science Advisory Board
SATs	Standard Assessment Tests
SBP	systolic blood pressure
SCE	sister chromatid exchange
Scna	α-synuclein
SD	standard deviation
SDN	sexually dimorphic nucleus
SE	standard error
Se	selenium
sec	second(s)
SEKI	Sequoia and Kings Canyon National Park, California
SEM	scanning electron microscopy; simultaneously extracted metal; standard error of the mean
SES	socioeconomic status
Sess.	Session
SFU	stacked filter unit(s)
SGA	small for gestational age
sGC	soluble guanylate cyclase
sGC-β1	soluble guanylate cyclase-beta 1
SGOT	serum glutamic oxaloacetic transaminase
SGPT	serum glutamic pyruvic transaminase
SHBG	sex hormone binding globulin
SHEDS	Stochastic Human Exposure and Dose (model)
SHM	Stockholm humic model
siRNA	small interfering RNA
SJW	silver jewelry workers
SLAMS	State and Local Air Monitoring Stations
SMC	smooth muscle cells

Acronym/Abbreviation	Meaning
SNAP-25	synaptosomal-associated protein 25
SNARE	soluble NSF attachment receptor
SNP	single-nucleotide polymorphism; sodium nitroprusside
SNS	sympathetic nervous system
SO	stratum oriens
SO_2	sulfur dioxide
So	south
SOC	superior olivary complex
SOD	superoxide dismutase
SOD1	superoxide dismutase-1
SOF	study of osteoporotic fractures
SOM	self-organizing map; soil organic matter
SP	spray painters
SP1, Sp1	specificity protein 1
SPM	suspended particulate matter
SPT	skin prick test
SREBP-2	sterol regulatory element binding protein-2
S. Rep.	Senate Report
SRIXE	synchrotron radiation induced X-ray emission
StAR	steroidogenic acute regulatory protein
STAT	signal transducer and activator of transcription
STAT3	signal transducer and activator of transcription 3
STAT5	signal transducer and activator of transcription 5
STD.	Standard
ST Interval	measured from the J point to the end of the T wave in an ECG
STN	Speciation Trends Network
Syb	synaptobrevin
Syn	synaptophysin
Syt	synaptotagmin
SZn	supplemental zinc

Acronym/Abbreviation	Meaning
T, t	time
T_3, T3	triiodothyronine
T_4, T4	thyroxine
$t_{1/2}$	half-life (-lives); time required to reduce the initial concentration by 50%
TBARS	thioBarbituric acid reactive substances; thiobarbituric acid-reactive species
T cell, T-cell	T lymphocyte
TE	trace elements
TEC	threshold effect concentrations
TEOM	tapered element oscillating microbalance, type of PM sampler
TF	ratio of the metal concentration in plant to that in soil; transferrin
TFIIIA	transcription factor IIIA
Tg	transgenic
TGF	transforming growth factor
TGF-β	β transforming growth factor
TGFβ1, TGF-β1	β1 transforming growth factor
TH	tyrosine hydroxylase
TH1, Th1	T-derived lymphocyte helper 1
TH2, Th2	T-derived lymphocyte helper 2
Th	T-helper lymphocyte
TIMP-1	tissue inhibitor of metalloproteinases-1
TIMS	thermal ionization mass spectrometry
TLC	Treatment of Lead-exposed Children (study)
T/LH	testosterone/luteinizing hormone - measure of Leydig cell function
TNF	tumor necrosis factor (e.g., TNF-α)
TNP-Ficoll	trinitrophenyl-Ficoll
TNP-OVA	trinitrophenyl-ovalbumin
TPR	total peripheral vascular resistance
TS	transferrin saturation

Acronym/Abbreviation	Meaning
TSH	thyroid stimulating hormone; total sulfhydryl
TSP	total suspended particles
TSS	total suspended solids
TXB_2	thromboxane
U	uranium
UA	urbanized area
UBL	urban boundary layer
UCL	urban canopy layer
UDDS	urban dynamometer driving schedule
UDPGT	uridine diphosphate (UDP)-glucuronosyltransferase(s)
UIUC	University of Illinois at Urbana Champaign
U.K.	United Kingdom
U.S.	United States of America
USC	U.S. Code
U.S. EPA	U.S. Environmental Protection Agency
USF	uptake slope factor
USGS	U.S. Geological Survey
USL	urban surface layer
UUDS	urban dynamic driving schedule
UV	ultraviolet radiation
UWM	Unit World Model
V	vanadium
V79	Chinese hamster lung cell line
VA	Veterans Administration
VAChAT	vesicular acetylcholine transporter
VAMP-2	vesicle-associated membrane protein-2
VA-NAS	Veterans Administration Normative Aging Study
VDAC	voltage-dependent anion channel
VDR	vitamin D receptor
VGAT	vesicular gamma aminobutyric acid (GABA) transporter
VGCC	voltage gated calcium channel(s)
VGLUT1	vesicular glutamate transporter 1
VIQ	verbal intelligence quotient (IQ)
VLPb	very low Pb
VMAT2	vesicular monoamine transporter-2
VO_4^{3-}	vanadate ion
VOC(s)	volatile organic compound(s)
vs., v.	versus
VSCC	very sharp cut cyclone
VSMC	vascular smooth muscle cells
WACAP	Western Airborne Contaminants Assessment Project
WBC	white blood cell
WCST	Wisconsin Card Sorting Test
WHAM	Windermere humic aqueous model
WHO	World Health Organization
WIAT	Wechsler Individual Achievement Test
WINS	well impactor ninety six
WISC	Wechsler Intelligence Scale for Children
WISC-R	Wechsler Intelligence Scale for Children-Revised
wk	week(s)
WML	white matter lesions
WPPSI-III	Wechsler Preschool and Primary Scales of Intelligence-III
WPPSI-R	Wechsler Preschool and Primary Scale of Intelligence-Revised
WRAT	Wide Range Achievement Test
W/S	winter/summer
WT	wild type
wt.	weight
XAFS	X-ray absorption fine structure
XANES	X-ray absorption near edge structure
XDH	xanthine dehydrogenase

Acronym/Abbreviation	Meaning
X_{ij}	observed hourly concentrations for time period i at site j
X_{ik}	observed hourly concentrations for time period i at site k
XPS	X-ray photoelectron spectroscopy
XRF	X-ray fluorescence
yr	year(s)
Zn	zinc
Zn^{2+}	zinc ion
ZPP	zirconium-potassium perchlorate; zinc protoporphyrin
Z-score	standard score

Preamble

Process of ISA Development

This preamble outlines the general process for developing an Integrated Science Assessment (ISA) including the framework for evaluating weight of evidence and drawing scientific conclusions and causal judgments. The ISA provides a concise review, synthesis, and evaluation of the most policy-relevant science to serve as a scientific foundation for the review of the National Ambient Air Quality Standards (NAAQS). The general process for NAAQS reviews is described at http://www.epa.gov/ttn/naaqs/review.html. Figure I depicts the general NAAQS review process and information for individual NAAQS reviews is available at www.epa.gov/ttn/naaqs. This preamble is a general discussion of the basic steps and criteria used in developing an ISA; for each ISA, specific details and considerations are included in the introductory section for that assessment.

The fundamental process for developing an ISA includes:

- literature searches;
- study selection;
- evaluation and integration of the evidence; and
- development of scientific conclusions and causal judgments.

An initial step in this process is publication of a call for information in the Federal Register that invites the public to provide information relevant to the assessment, such as new or recent publications on health or welfare[1] effects of the pollutant, or from atmospheric and exposure sciences fields. The U.S. Environmental Protection Agency (EPA) maintains an ongoing literature search process for identification of relevant scientific studies published since the last review of the NAAQS. Search strategies are designed for pollutants and scientific disciplines and iteratively modified to optimize identification of pertinent publications. Papers are identified for inclusion in several additional ways: specialized searches on specific topics; independent review of tables of contents for journals in which relevant papers may be published; independent identification of relevant literature by expert scientists; review of citations in previous assessments and identification by the public and the Clean Air Scientific Advisory Committee (CASAC) during the external review process. This literature search and study

[1] Welfare effects as defined in Clean Air Act (CAA) Section 302(h) [42 U.S.C. 7602(h)] include, but are not limited to, “effects on soils, water, crops, vegetation, man-made materials, animals, wildlife, weather, visibility and climate, damage to and deterioration of property, and hazards to transportation, as well as effects on economic values and on personal comfort and well-being.”

selection process is depicted in Figure II. Publications considered for inclusion in the ISA are added to the Health and Environmental Research Online (HERO) database developed by EPA (http://hero.epa.gov/); the references in the ISA include a hyperlink to the database.

Studies that have undergone scientific peer review and have been published or accepted for publication and reports that have undergone review are considered for inclusion in the ISA. Analyses conducted by EPA using publicly available data are also considered for inclusion in the ISA. All relevant epidemiologic, controlled human exposure, toxicological, and ecological and welfare effects studies published since the last review are considered, including those related to exposure-response relationships, mode(s) of action (MOA), and potentially at-risk populations and lifestages. Studies on atmospheric chemistry, environmental fate and transport, dosimetry, toxicokinetics and exposure are also considered for inclusion in the document, as well as analyses of air quality and emissions data. References that were considered for inclusion in a specific ISA can be found using the HERO website (http://hero.epa.gov).

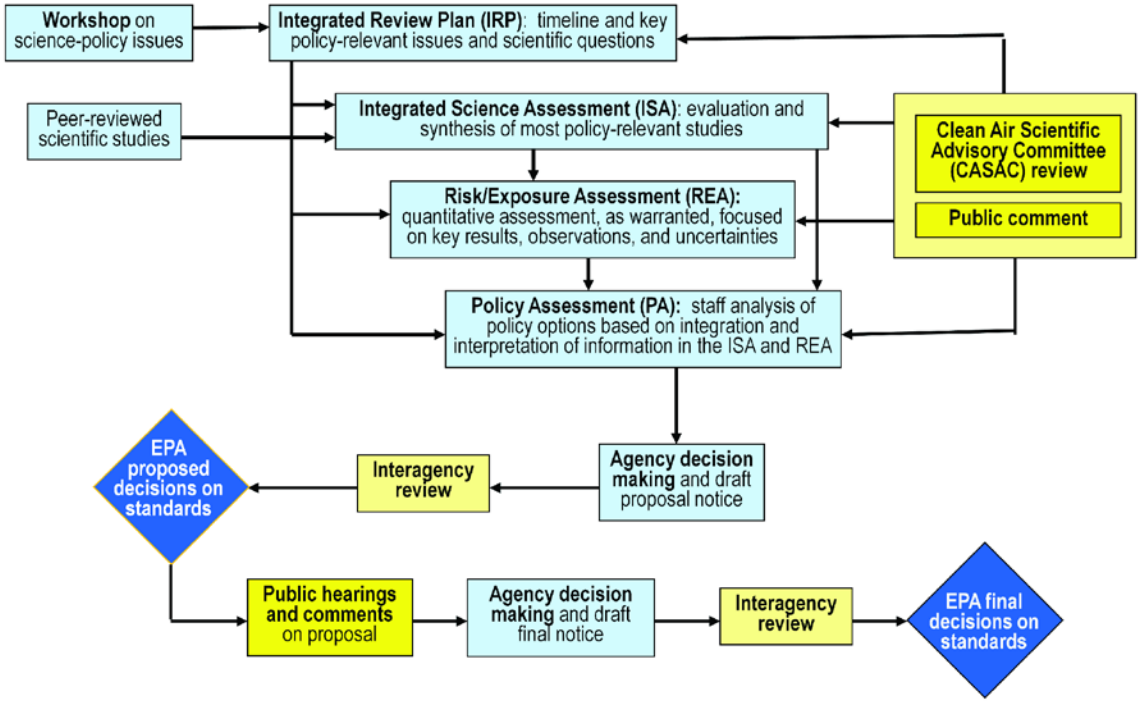

Figure I **Illustration of the key steps in the process of the review of National Ambient Air Quality Standards.**

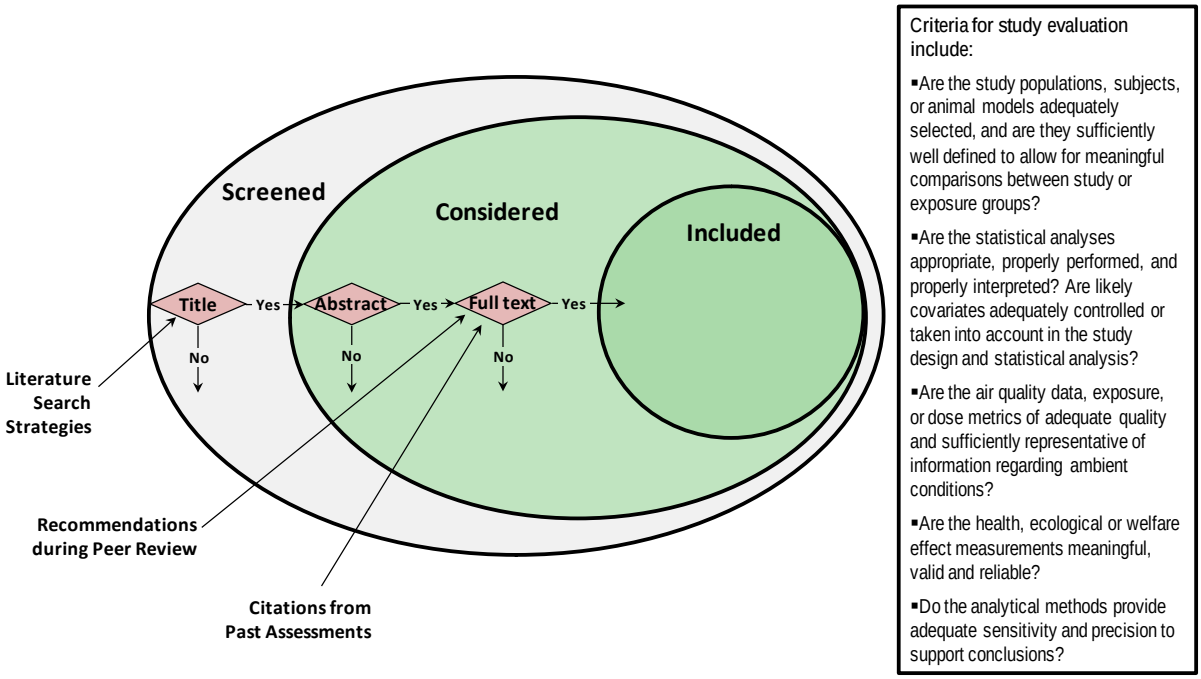

Figure II **Illustration of processes for literature search and study selection used for development of ISAs.**

Each ISA builds upon the conclusions of previous assessments for the pollutant under review. EPA focuses on peer reviewed literature published following the completion of the previous review and on any new interpretations of previous literature, integrating the results of recent scientific studies with previous findings. Important earlier studies may be discussed in detail to reinforce key concepts and conclusions or for reinterpretation in light of newer data. Earlier studies also are the primary focus in some areas of the document where research efforts have subsided, or if these earlier studies remain the definitive works available in the literature.

Selection of studies for inclusion in the ISA is based on the general scientific quality of the study, and consideration of the extent to which the study is informative and policy-relevant. Policy-relevant and informative studies include those that provide a basis for or describe the relationship between the criteria pollutant and effects, including studies that offer innovation in method or design and studies that reduce uncertainty on critical issues, such as analyses of confounding or effect modification by copollutants or other variables, analyses of concentration-response or dose-response relationships, or analyses related to time between exposure and response. Emphasis is placed on studies that examine effects associated with pollutant concentrations relevant to current population and ecosystem exposures, and particularly those pertaining to concentrations currently found in ambient

air. Other studies are included if they contain unique data, such as a previously unreported effect or MOA for an observed effect, or examine multiple concentrations to elucidate exposure-response relationships. In general, in assessing the scientific quality and relevance of health and welfare effects studies, the following considerations have been taken into account when selecting studies for inclusion in the ISA.

- Are the study populations, subjects, or animal models adequately selected, and are they sufficiently well defined to allow for meaningful comparisons between study or exposure groups?
- Are the statistical analyses appropriate, properly performed, and properly interpreted? Are likely covariates adequately controlled or taken into account in the study design and statistical analysis?
- Are the air quality data, exposure, or dose metrics of adequate quality and sufficiently representative of information regarding ambient conditions?
- Are the health, ecological or welfare effect measurements meaningful, valid and reliable?
- Do the analytical methods provide adequate sensitivity and precision to support conclusions?

Additional considerations are specific to particular disciplines. In selecting epidemiologic studies, EPA considers whether a given study: (1) presents information on associations with short- or long-term pollutant exposures at or near conditions relevant to ambient exposures; (2) addresses potential confounding by other pollutants; (3) assesses potential effect modifiers; (4) evaluates health endpoints and populations not previously extensively researched; and (5) evaluates important methodological issues related to interpretation of the health evidence (e.g., lag or time period between exposure and effects, model specifications, thresholds, mortality displacement).

Considerations for the selection of research evaluating controlled human exposure or animal toxicological studies include a focus on studies conducted using relevant pollutant exposures. For both types of studies, relevant pollutant exposures are considered to be those generally within one or two orders of magnitude of ambient concentrations. Studies in which higher doses were used may also be considered if they provide information relevant to understanding MOA or mechanisms, as noted below.

Evaluation of controlled human exposure studies focuses on those that approximated expected human exposure conditions in terms of concentration and duration. Studies should include control exposures to filtered air, as appropriate. In the selection of controlled human exposure studies, emphasis is placed on studies that: (1) investigate potentially at-risk populations and lifestages such as people with asthma or cardiovascular diseases, children or older adults; (2) address issues such as concentration-

response or time-course of responses; and (3) have sufficient statistical power to assess findings.

Review of the animal toxicological evidence focuses on studies that approximate expected human dose conditions, which vary depending on the dosimetry, toxicokinetics and biological sensitivity of the particular laboratory animal species or strains studied. Emphasis is placed on studies that: (1) investigate animal models of disease that can provide information on populations potentially at increased risk of effects; (2) address issues such as concentration-response or time-course of responses; and (3) have sufficient statistical power to assess findings. Due to resource constraints on exposure duration and numbers of animals tested, animal studies typically utilize high-concentration exposures to acquire data relating to mechanisms and assure a measurable response. Emphasis is placed on studies using doses or concentrations generally within 1-2 orders of magnitude of current levels. Studies with higher concentration exposures or doses are considered to the extent that they provide useful information to inform understanding of interspecies differences and differences between healthy and at-risk human populations. Results from in vitro studies may also be included if they provide mechanistic insight or further support for results demonstrated in vivo.

These criteria provide benchmarks for evaluating various studies and for focusing on the policy-relevant studies in assessing the body of health, ecological and welfare effects evidence. As stated initially, the intent of the ISA is to provide a concise review, synthesis, and evaluation of the most policy-relevant science to serve as a scientific foundation for the review of the NAAQS, not extensive summaries of all health, ecological and welfare effects studies for a pollutant. Of most relevance for inclusion of studies is whether they provide useful qualitative or quantitative information on exposure-effect or exposure-response relationships for effects associated with pollutant exposures at doses or concentrations relevant to ambient conditions that can inform decisions on whether to retain or revise the standards.

The general process for ISA development is illustrated in Figure III. In developing an ISA, EPA reviews and summarizes the evidence from: studies of atmospheric sciences; human exposure, animal toxicological, controlled human exposure and epidemiologic studies; and studies of ecological and welfare effects. In the process of developing the first draft ISA, EPA may convene a peer input meeting in which the scientific content of preliminary draft materials is reviewed to ensure that the ISA is up to date and is focused on the most policy-relevant findings, and to assist EPA with integration of evidence within and across disciplines. EPA integrates the evidence from across scientific disciplines or study types and characterizes the weight of evidence for relationships between the pollutant and various outcomes. The integration of evidence on health, and

ecological or welfare effects, involves collaboration between scientists from various disciplines. As an example, an evaluation of health effects evidence would include the integration of the results from epidemiologic, controlled human exposure, and toxicological studies, and application of the causal framework (described below) to draw conclusions. Integration of results on health or ecological effects that are logically or mechanistically connected (e.g., a spectrum of effects on the respiratory system) informs judgments of causality. Using the causal framework described in the following section, EPA scientists consider aspects such as strength, consistency, coherence, and biological plausibility of the evidence, and develop causality determinations on the nature of the relationships. Causality determinations often entail an iterative process of review and evaluation of the evidence. Two drafts of the ISA are typically released for review by the CASAC and the public, and comments received on the characterization of the science as well as the implementation of the causal framework are carefully considered in revising and completing the final ISA.

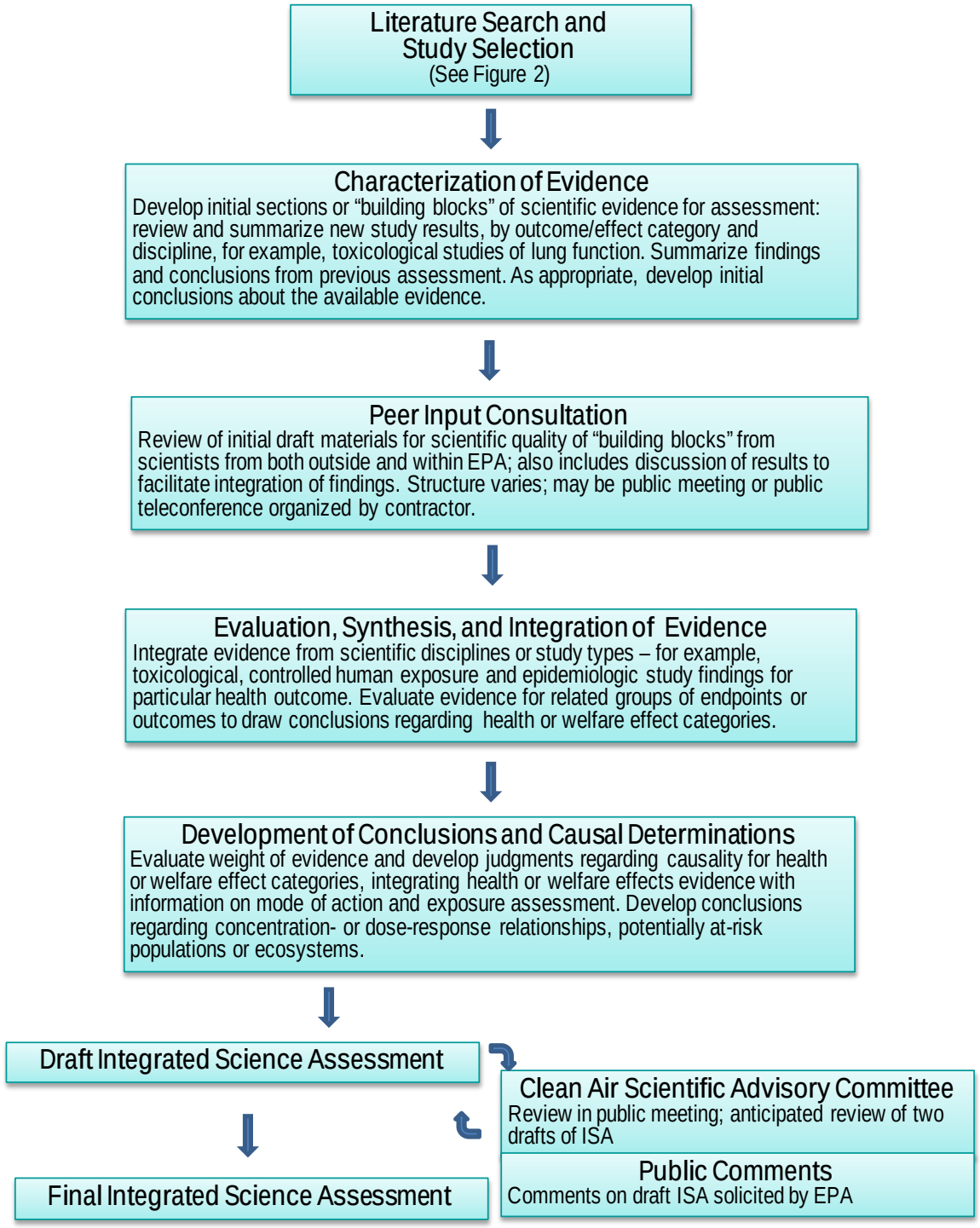

Figure III Characterization of the general process of ISA development.

EPA Framework for Causal Determination

EPA has developed a consistent and transparent basis for integration of scientific evidence and evaluation of the causal nature of air pollution-related health or welfare effects for use in developing ISAs. The framework described below establishes uniform language concerning causality and brings more specificity to the findings. This standardized language was drawn from sources across the federal government and wider scientific community, especially the National Academy of Sciences (NAS) Institute of Medicine (IOM) document, *Improving the Presumptive Disability Decision-Making Process for Veterans* (2008), a comprehensive report on evaluating causality. This framework:

- describes the kinds of scientific evidence used in establishing a general causal relationship between exposure and health effects;
- characterizes the process for integration and evaluation of evidence necessary to reach a conclusion about the existence of a causal relationship;
- identifies issues and approaches related to uncertainty; and
- provides a framework for classifying and characterizing the weight of evidence in support of a general causal relationship.

Approaches to assessing the separate and combined lines of evidence (e.g., epidemiologic, controlled human exposure, and animal toxicological studies) have been formulated by a number of regulatory and science agencies, including the IOM of the NAS (2008), International Agency for Research on Cancer (IARC) (2006b), U.S. EPA (2005c), and Centers for Disease Control and Prevention (CDC) (2004). Causal inference criteria have also been described for ecological effects evidence (U.S. EPA, 1998; Fox, 1991). These formalized approaches offer guidance for assessing causality. The frameworks are similar in nature, although adapted to different purposes, and have proven effective in providing a uniform structure and language for causal determinations.

Evaluating Evidence for Inferring Causation

The 1964 Surgeon General's report defined "cause" as a "significant, effectual relationship between an agent and an associated disorder or disease in the host" (HEW, 1964). More generally, a cause is defined as an agent that brings about an effect or a result. An association is the statistical relationship among variables; alone, however, it is insufficient proof of a causal relationship between an exposure and a health outcome. Unlike an association, a causal claim supports the creation of counterfactual claims, that is, a claim about what the world would have been like under different or changed circumstances [Samet and Bodurow, eds. (IOM, 2008)].

Many of the health and environmental outcomes reported in these studies have complex etiologies. Diseases such as asthma, coronary heart disease (CHD) or cancer are typically initiated by multiple agents. Outcomes depend on a variety of factors, such as age, genetic susceptibility, nutritional status, immune competence, and social factors (IOM, 2008; Gee and Payne-Sturges, 2004). Effects on ecosystems are often also multifactorial with a complex web of causation. Further, exposure to a combination of agents could cause synergistic or antagonistic effects. Thus, the observed risk may represent the net effect of many actions and counteractions.

Scientific findings incorporate uncertainty. "Uncertainty" can be defined as having limited knowledge to exactly describe an existing state or future outcome, e.g., the lack of knowledge about the correct value for a specific measure or estimate. Uncertainty analysis may be qualitative or quantitative in nature. In many cases, the analysis is qualitative, and can include professional judgment or inferences based on analogy with similar situations. Quantitative uncertainty analysis may include use of simple measures (e.g., ranges) and analytical techniques. Quantitative uncertainty analysis might progress to more complex measures and techniques, if needed for decision support. Various approaches to evaluating uncertainty include classical statistical methods, sensitivity analysis, or probabilistic uncertainty analysis, in order of increasing complexity and data requirements. However, data may not be available for all aspects of an assessment and those data that are available may be of questionable or unknown quality. Ultimately, the assessment is based on a number of assumptions with varying degrees of uncertainty. The ISA generally evaluates uncertainties qualitatively in assessing the evidence from across studies; in some situations quantitative analysis approaches, such as meta-regression, may be used.

Publication bias is a source of uncertainty regarding the magnitude of health risk estimates. It is well understood that studies reporting non-null findings are more likely to be published than reports of null findings. Publication bias can result in overestimation of effect estimate sizes (Ioannidis, 2008). For example, effect estimates from single-city epidemiologic studies have been found to be generally larger than those from multicity studies which is an indication of publication bias in that null or negative single-city results may be reported in a multicity analyses but might not be published independently (Bell et al., 2005).

Consideration of Evidence from Scientific Disciplines

Moving from association to causation involves the elimination of alternative explanations for the association. The ISA focuses on evaluation of the findings from the body of evidence, drawing upon the results of all studies determined to meet the criteria described previously. Causality determinations are based on the evaluation, integration, and synthesis of evidence from across scientific disciplines. The relative importance of different types of evidence varies by pollutant or assessment, as does the availability of different types of evidence for causality determination. Three general types of studies inform consideration of human health effects: controlled human exposure, epidemiologic and toxicological studies. Evidence on ecological or welfare effects may be drawn from a variety of experimental approaches (e.g., greenhouse, laboratory, and field) and numerous disciplines (e.g., community ecology, biogeochemistry and paleontological/historical reconstructions).

Direct evidence of a relationship between pollutant exposures and human health effects comes from controlled human exposure studies. Such studies experimentally evaluate the health effects of administered exposures in human volunteers under highly controlled laboratory conditions. Also referred to as human clinical studies, these experiments allow investigators to expose subjects to known concentrations of air pollutants under carefully regulated environmental conditions and activity levels. These studies provide important information on the biological plausibility of associations observed in epidemiologic studies. In some instances, controlled human exposure studies can also be used to characterize concentration-response relationships at pollutant concentrations relevant to ambient conditions. Controlled human exposures are typically conducted using a randomized crossover design, with subjects exposed both to the pollutant and a clean air control. In this way, subjects serve as their own experimental controls, effectively limiting the variance associated with many potential confounders. Considerations for evaluating controlled human study findings include the generally small sample size and short exposure time used in experimental studies, and that severe health outcomes are not assessed. By experimental design, controlled human exposure studies are structured to evaluate physiological or biomolecular outcomes in response to exposure to a specific air pollutant and/or combination of pollutants. In addition, the study design generally precludes inclusion of subjects with serious health conditions, and therefore the results often cannot be generalized to an entire population. Although some controlled human exposure studies have included health-compromised individuals such as those with respiratory or cardiovascular disease, these individuals may also be relatively healthy and may not represent the most sensitive individuals in the population. Thus, observed effects in these studies may underestimate the response in certain populations. In addition, the

study design is limited to exposures and endpoints that are not expected to result in severe health outcomes.

Epidemiologic studies provide important information on the associations between health effects and exposure of human populations to ambient air pollution. In epidemiologic or observational studies of humans, the investigator generally does not control exposures or intervene with the study population. Broadly, observational studies can describe associations between exposures and effects. These studies fall into several categories: e.g., cross-sectional, prospective cohort, panel, and time-series studies. Cross-sectional studies generally use health outcome, exposure and covariate data available at the community level (e.g., annual mortality rates and pollutant concentrations), but do not have individual-level data. Prospective cohort studies have some data collected at the individual level, generally health outcome data, and in some cases individual-level data on exposure and covariates are collected. Time-series studies evaluate the relationship for changes in a health outcome with changes in exposure indicators, such as an association between daily changes in mortality with air pollution. Panel studies include repeated measurements of health outcomes, such as respiratory symptoms or heart rhythm variability, at the individual level. “Natural experiments” offer the opportunity to investigate changes in health related to a change in exposure, such as closure of a pollution source.

In evaluating epidemiologic studies, consideration of many study design factors and issues must be taken into account to properly inform their interpretation. One key consideration is evaluation of the potential contribution of the pollutant to a health outcome when it is a component of a complex air pollutant mixture. Reported effect estimates in epidemiologic studies may reflect (1) independent effects on health outcomes; (2) effects of the pollutant acting as an indicator of a copollutant or a complex ambient air pollution mixture; and (3) effects resulting from interactions between that pollutant and copollutants.

In the evaluation of epidemiologic evidence, one important consideration is potential confounding. Confounding is “… a confusion of effects. Specifically, the apparent effect of the exposure of interest is distorted because the effect of an extraneous factor is mistaken for or mixed with the actual exposure effect (which may be null)” (Rothman and Greenland, 1998). One approach to remove spurious associations due to possible confounders is to control for characteristics that may differ between exposed and unexposed persons; this is frequently termed “adjustment.” Scientific judgment is needed to evaluate likely sources and extent of confounding, together with consideration of how well the existing constellation of study designs, results, and analyses address the potential for erroneous inferences. A confounder is associated with both the exposure and the

effect; for example, confounding can occur between correlated pollutants that are associated with the same effect.

Several statistical methods are available to detect and control for potential confounders; however, none of these methods is completely satisfactory. Multivariable regression models constitute one tool for estimating the association between exposure and outcome after adjusting for characteristics of participants that might confound the results. The use of multipollutant regression models has been the prevailing approach for controlling potential confounding by copollutants in air pollution health effects studies. Finding the likely causal pollutant from multipollutant regression models is made difficult by the possibility that one or more air pollutants may be acting as a surrogate for an unmeasured or poorly measured pollutant or for a particular mixture of pollutants. In addition, pollutants may independently exert effects on the same system; for example, several pollutants may be associated with respiratory effect through either the same or different modes of action. The number and degree of diversity of covariates, as well as their relevance to the potential confounders, remain matters of scientific judgment. Despite these limitations, the use of multipollutant models is still the prevailing approach employed in most air pollution epidemiologic studies and provides some insight into the potential for confounding or interaction among pollutants.

Confidence that unmeasured confounders are not producing the findings is increased when multiple studies are conducted in various settings using different subjects or exposures, each of which might eliminate another source of confounding from consideration. For example, multicity studies can provide insight on potential confounding through the use of a consistent method to analyze data from across locations with different levels of copollutants and other covariates. Intervention studies, because of their quasi-experimental nature, can be particularly useful in characterizing causation.

Another important consideration in the evaluation of epidemiologic evidence is effect modification, which occurs when the effect differs between subgroups or strata; for example, effect estimates that vary by age group or potential risk factor. As stated by Rothman and Greenland (1998):

> "Effect-measure modification differs from confounding in several ways. The main difference is that, whereas confounding is a bias that the investigator hopes to prevent or remove from the effect estimate, effect-measure modification is a property of the effect under study … In epidemiologic analysis one tries to eliminate confounding but one tries to detect and estimate effect-measure modification."

When a risk factor is a confounder, it is the true cause of the association observed between the exposure and the outcome; when a risk factor is an effect modifier, it changes the magnitude of the association between the exposure and the outcome in

stratified analyses. For example, the presence of a preexisting disease or indicator of low socioeconomic status may act as effect modifiers if they are associated with increased risk of effects related to air pollution exposure. It is often possible to stratify the relationship between health outcome and exposure by one or more of these potential effect modifiers. For variables that modify the association, effect estimates in each stratum will be different from one another and different from the overall estimate, indicating a different exposure-response relationship may exist in populations represented by these variables.

Exposure measurement error, which refers to the uncertainty associated with the exposure metrics used to represent exposure of an individual or population, can be an important contributor to uncertainty in air pollution epidemiologic study results. Exposure error can influence observed epidemiologic associations between ambient pollutant concentrations and health outcomes by biasing effect estimates toward or away from the null and widening confidence intervals around those estimates (Zeger et al., 2000). There are several components that contribute to exposure measurement error in air pollution epidemiologic studies, including the difference between true and measured ambient concentrations, the difference between average personal exposure to ambient pollutants and ambient concentrations at central monitoring sites, and the use of average population exposure rather than individual exposure estimates. Factors that could influence exposure estimates include nonambient sources of exposure, topography of the natural and built environment, meteorology, measurement errors, time-location-activity patterns, and the extent to which ambient pollutants penetrate indoor environments. The importance of exposure error varies with study design and is dependent on the spatial and temporal aspects of the design.

The third main type of health effects evidence, animal toxicological studies, provides information on the pollutant's biological action under controlled and monitored exposure circumstances. Taking into account physiological differences of the experimental species from humans, these studies inform characterization of health effects of concern, exposure-response relationships and MOAs. Further, animal models can inform determinations of at-risk populations. These studies evaluate the effects of exposures to a variety of pollutants in a highly controlled laboratory setting and allow exploration of toxicological pathways or mechanisms by which a pollutant may cause effects. Understanding the biological mechanisms underlying various health outcomes can prove crucial in establishing or negating causality. In the absence of human studies data, extensive, well-conducted animal toxicological studies can support determinations of causality, if the evidence base indicates that similar responses are expected in humans under ambient exposure conditions.

Interpretations of animal toxicological studies are affected by limitations associated with extrapolation between animal and human responses. The differences between humans and other species have to be taken into consideration, including metabolism, hormonal regulation, breathing pattern, and differences in lung structure and anatomy. Also, in spite of a high degree of homology and the existence of a high percentage of orthologous genes across humans and rodents (particularly mice), extrapolation of molecular alterations at the gene level is complicated by species-specific differences in transcriptional regulation. Given these differences, there are uncertainties associated with quantitative extrapolations of observed pollutant-induced pathophysiological alterations between laboratory animals and humans, as those alterations are under the control of widely varying biochemical, endocrine, and neuronal factors.

For ecological effects assessment, both laboratory and field studies (including field experiments and observational studies) can provide useful data for causality determination. Because conditions can be controlled in laboratory studies, responses may be less variable and smaller differences may be easier to detect. However, the control conditions may limit the range of responses (e.g., animals may not be able to seek alternative food sources) or incompletely reflect pollutant bioavailability, so they may not reflect responses that would occur in the natural environment. In addition, larger-scale processes are difficult to reproduce in the laboratory.

Field observational studies measure biological changes in uncontrolled situations, and describe an association between a disturbance and an ecological effect. Field data can provide important information for assessments of multiple stressors or where site-specific factors significantly influence exposure. They are also often useful for analyses of larger geographic scales and higher levels of biological organization. However, because conditions are not controlled, variability is expected to be higher and differences harder to detect. Field surveys are most useful for linking stressors with effects when stressor and effect levels are measured concurrently. The presence of confounding factors can make it difficult to attribute observed effects to specific stressors.

Some studies are considered “intermediate” and are categorized as being between laboratory and field are studies. Some use environmental media collected from the field to examine the responses in the laboratory. Others are experiments that are performed in the natural environment while controlling for some, but not all, of the environmental conditions (i.e., mesocosm studies). This type of study in manipulated natural environments can be considered a hybrid between a field experiment and laboratory study since some aspects are performed under controlled conditions but others are not. They make it possible to observe community and/or ecosystem dynamics, and provide strong

evidence for causality when combined with findings of studies that have been made under more controlled conditions.

Application of Framework for Causal Determination

In its evaluation and integration of the scientific evidence on health or welfare effects of criteria pollutants, EPA determines the weight of evidence in support of causation and characterizes the strength of any resulting causal classification. EPA also evaluates the quantitative evidence and draws scientific conclusions, to the extent possible, regarding the concentration-response relationships and the loads to ecosystems, exposures, doses or concentrations, exposure duration, and pattern of exposures at which effects are observed.

To aid judgment, various "aspects"[1] of causality have been discussed by many philosophers and scientists. The 1964 Surgeon General's report on tobacco smoking discussed criteria for the evaluation of epidemiologic studies, focusing on consistency, strength, specificity, temporal relationship, and coherence (HEW, 1964). Sir Austin Bradford Hill (Hill, 1965) articulated aspects of causality in epidemiology and public health that have been widely used (IOM, 2008; IARC, 2006b; U.S. EPA, 2005c; CDC, 2004). These aspects (Hill, 1965) have been modified (Table I) for use in causal determinations specific to health and welfare effects for pollutant exposures (U.S. EPA, 2009a).[2] Although these aspects provide a framework for assessing the evidence, they do not lend themselves to being considered in terms of simple formulas or fixed rules of evidence leading to conclusions about causality (Hill, 1965). For example, one cannot simply count the number of studies reporting statistically significant results or statistically nonsignificant results and reach credible conclusions about the relative weight of the evidence and the likelihood of causality. Rather, these aspects provide a framework for systematic appraisal of the body of evidence, informed by peer and public comment and advice, which includes weighing alternative views on controversial issues. In addition, it is important to note that the aspects in Table I cannot be used as a strict checklist, but rather to determine the weight of the evidence for inferring causality. In particular, not meeting one or more of the principles does not automatically preclude a determination of causality [see discussion in (CDC, 2004)].

[1] The "aspects" described by Sir Austin Bradford Hill (Hill, 1965) have become, in the subsequent literature, more commonly described as "criteria." The original term "aspects" is used here to avoid confusion with "criteria" as it is used, with different meaning, in the Clean Air Act.

[2] The Hill aspects were developed for interpretation of epidemiologic results. They have been modified here for use with a broader array of data, i.e., epidemiologic, controlled human exposure, ecological, and animal toxicological studies, as well as in vitro data, and to be more consistent with EPA's Guidelines for Carcinogen Risk Assessment.

Table I Aspects to aid in judging causality.

Aspect	Description
Consistency of the observed association	An inference of causality is strengthened when a pattern of elevated risks is observed across several independent studies. The reproducibility of findings constitutes one of the strongest arguments for causality. If there are discordant results among investigations, possible reasons such as differences in exposure, confounding factors, and the power of the study are considered.
Coherence	An inference of causality from one line of evidence (e.g., epidemiologic, clinical, or animal studies) may be strengthened by other lines of evidence that support a cause-and-effect interpretation of the association. Evidence on ecological or welfare effects may be drawn from a variety of experimental approaches (e.g., greenhouse, laboratory, and field) and subdisciplines of ecology (e.g., community ecology, biogeochemistry, and paleontological/historical reconstructions). The coherence of evidence from various fields greatly adds to the strength of an inference of causality. In addition, there may be coherence in demonstrating effects across multiple study designs or related health endpoints within one scientific line of evidence.
Biological plausibility.	An inference of causality tends to be strengthened by consistency with data from experimental studies or other sources demonstrating plausible biological mechanisms. A proposed mechanistic linking between an effect and exposure to the agent is an important source of support for causality, especially when data establishing the existence and functioning of those mechanistic links are available.
Biological gradient (exposure-response relationship)	A well-characterized exposure-response relationship (e.g., increasing effects associated with greater exposure) strongly suggests cause and effect, especially when such relationships are also observed for duration of exposure (e.g., increasing effects observed following longer exposure times).
Strength of the observed association	The finding of large, precise risks increases confidence that the association is not likely due to chance, bias, or other factors. However, it is noted that a small magnitude in an effect estimate may represent a substantial effect in a population.
Experimental evidence	Strong evidence for causality can be provided through “natural experiments” when a change in exposure is found to result in a change in occurrence or frequency of health or welfare effects.
Temporal relationship of the observed association	Evidence of a temporal sequence between the introduction of an agent, and appearance of the effect, constitutes another argument in favor of causality.
Specificity of the observed association	Evidence linking a specific outcome to an exposure can provide a strong argument for causation. However, it must be recognized that rarely, if ever, does exposure to a pollutant invariably predict the occurrence of an outcome, and that a given outcome may have multiple causes.
Analogy	Structure activity relationships and information on the agent’s structural analogs can provide insight into whether an association is causal. Similarly, information on mode of action for a chemical, as one of many structural analogs, can inform decisions regarding likely causality.

Determination of Causality

In the ISA, EPA assesses the body of relevant literature, building upon evidence available during previous NAAQS reviews, to draw conclusions on the causal relationships between relevant pollutant exposures and health or environmental effects. ISAs use a five-level hierarchy that classifies the weight of evidence for causation[1]. In developing this hierarchy, EPA has drawn on the work of previous evaluations, most prominently the IOM's *Improving the Presumptive Disability Decision-Making Process for Veterans* [Samet and Bodurow, eds. (IOM, 2008), EPA's Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2005c), and the U.S. Surgeon General's smoking report (CDC, 2004). This weight of evidence evaluation is based on integration of findings from various lines of evidence from across the health and environmental effects disciplines. These separate judgments are integrated into a qualitative statement about the overall weight of the evidence and causality. The five descriptors for causal determination are described in Table II.

Determination of causality involves the evaluation and integration of evidence for different types of health, ecological or welfare effects associated with short- and long-term exposure periods. In making determinations of causality, evidence is evaluated for major outcome categories or groups of related endpoints (e.g., respiratory effects, vegetation growth), integrating evidence from across disciplines, and evaluating the coherence of evidence across a spectrum of related endpoints to draw conclusions regarding causality. In discussing the causal determination, EPA characterizes the evidence on which the judgment is based, including strength of evidence for individual endpoints within the outcome category or group of related endpoints.

In drawing judgments regarding causality for the criteria air pollutants, the ISA focuses on evidence of effects in the range of relevant pollutant exposures or doses, and not on determination of causality at any dose. Emphasis is placed on evidence of effects at doses (e.g., blood Pb concentration) or exposures (e.g., air concentrations) that are relevant to, or somewhat above, those currently experienced by the population. The extent to which studies of higher concentrations are considered varies by pollutant and major outcome category, but generally includes those with doses or exposures in the range of one to two orders of magnitude above current or ambient conditions. Studies that use higher doses or exposures may also be considered to the extent that they provide useful information to inform understanding of mode of action, interspecies differences, or factors that may increase risk of effects for a population. Thus, a causality determination is based on

[1] The Center for Disease Control (CDC) and IOM frameworks use a four-category hierarchy for the strength of the evidence. A five-level hierarchy is used here to be consistent with the EPA Guidelines for Carcinogen Risk Assessment and to provide a more nuanced set of categories.

weight of evidence evaluation for health, ecological or welfare effects, focusing on the evidence from exposures or doses generally ranging from current levels to one or two orders of magnitude above current levels.

In addition, EPA evaluates evidence relevant to understand the quantitative relationships between pollutant exposures and health, ecological or welfare effects. This includes evaluation of the form of concentration-response or dose-response relationships and, to the extent possible, drawing conclusions on the levels at which effects are observed. The ISA also draws scientific conclusions regarding important exposure conditions for effects and populations that may be at greater risk for effects, as described in the following section.

Table II Weight of evidence for causal determination.

Causal Determination	Health Effects	Ecological and Welfare Effects
Causal relationship	Evidence is sufficient to conclude that there is a causal relationship with relevant pollutant exposures (i.e., doses or exposures generally within one to two orders of magnitude of current levels). That is, the pollutant has been shown to result in health effects in studies in which chance, bias, and confounding could be ruled out with reasonable confidence. For example: a) controlled human exposure studies that demonstrate consistent effects; or b) observational studies that cannot be explained by plausible alternatives or are supported by other lines of evidence (e.g., animal studies or mode of action information). Evidence includes multiple high-quality studies	Evidence is sufficient to conclude that there is a causal relationship with relevant pollutant exposures i.e., doses or exposures generally within one to two orders of magnitude of current levels). That is, the pollutant has been shown to result in effects in studies in which chance, bias, and confounding could be ruled out with reasonable confidence. Controlled exposure studies (laboratory or small- to medium-scale field studies) provide the strongest evidence for causality, but the scope of inference may be limited. Generally, determination is based on multiple studies conducted by multiple research groups, and evidence that is considered sufficient to infer a causal relationship is usually obtained from the joint consideration of many lines of evidence that reinforce each other.
Likely to be a causal relationship	Evidence is sufficient to conclude that a causal relationship is likely to exist with relevant pollutant exposures, but important uncertainties remain. That is, the pollutant has been shown to result in health effects in studies in which chance and bias can be ruled out with reasonable confidence but potential issues remain. For example: a) observational studies show an association, but copollutant exposures are difficult to address and/or other lines of evidence (controlled human exposure, animal, or mode of action information) are limited or inconsistent; or b) animal toxicological evidence from multiple studies from different laboratories that demonstrate effects, but limited or no human data are available. Evidence generally includes multiple high-quality studies.	Evidence is sufficient to conclude that there is a likely causal association with relevant pollutant exposures. That is, an association has been observed between the pollutant and the outcome in studies in which chance, bias, and confounding are minimized, but uncertainties remain. For example, field studies show a relationship, but suspected interacting factors cannot be controlled, and other lines of evidence are limited or inconsistent. Generally, determination is based on multiple studies in multiple research groups.
Suggestive of a causal relationship	Evidence is suggestive of a causal relationship with relevant pollutant exposures, but is limited. For example, (a) at least one high-quality epidemiologic study shows an association with a given health outcome but the results of other studies are inconsistent; or (b) a well-conducted toxicological study, such as those conducted in the National Toxicology Program (NTP), shows effects in animal species,	Evidence is suggestive of a causal relationship with relevant pollutant exposures, but chance, bias and confounding cannot be ruled out. For example, at least one high-quality study shows an effect, but the results of other studies are inconsistent.
Inadequate to infer a causal relationship	Evidence is inadequate to determine that a causal relationship exists with relevant pollutant exposures. The available studies are of insufficient quantity, quality, consistency, or statistical power to permit a conclusion regarding the presence or absence of an effect.	The available studies are of insufficient quality, consistency, or statistical power to permit a conclusion regarding the presence or absence of an effect.
Not likely to be a causal relationship	Evidence is suggestive of no causal relationship with relevant pollutant exposures. Several adequate studies, covering the full range of levels of exposure that human beings are known to encounter and considering at-risk populations, are mutually consistent in not showing an effect at any level of exposure.	Several adequate studies, examining relationships with relevant exposures, are consistent in failing to show an effect at any level of exposure.

Quantitative Relationships: Effects on Human Populations

Once a determination is made regarding the causal relationship between the pollutant and outcome category, important questions regarding quantitative relationships include:

- What is the concentration-response, exposure-response, or dose-response relationship in the human population?
- What is the interrelationship between incidence and severity of effect?
- What exposure conditions (dose or exposure, duration and pattern) are important?
- What populations and lifestages appear to be differentially affected (i.e., more at risk of experiencing effects)?

In order to address these questions, the entirety of quantitative evidence is evaluated to characterize pollutant concentrations and exposure durations at which effects were observed for exposed populations, including populations and lifestages potentially at increased risk. To accomplish this, evidence is considered from multiple and diverse types of studies, and a study or set of studies that best approximates the concentration-response relationships between health outcomes and the pollutant may be identified. Controlled human exposure studies provide the most direct and quantifiable exposure-response data on the human health effects of pollutant exposures. To the extent available, the ISA evaluates results from epidemiologic studies that characterize the form of relationships between the pollutant and health outcomes and draws conclusions on the shape of these relationships. Animal data may also inform evaluation of concentration-response relationships, particularly relative to MOAs and characteristics of at-risk populations.

An important consideration in characterizing the public health impacts associated with exposure to a pollutant is whether the concentration-response relationship is linear across the range of concentrations or if nonlinear relationships exist along any part of this range. The shape of the concentration-response curve at and below the level of the current standards is of particular interest. Various sources of variability and uncertainty, such as low data density in the lower concentration range, possible influence of exposure measurement error, and variability between individuals in susceptibility to air pollution health effects, tend to smooth and “linearize” the concentration-response function, and thus can obscure the existence of a threshold or nonlinear relationship. Since individual thresholds vary from person to person due to individual differences such as genetic level susceptibility or preexisting disease conditions (and even can vary from one time to another for a given person), it can be difficult to demonstrate that a threshold exists in a population study. These sources of variability and uncertainty may explain why the available human data at ambient concentrations for some environmental pollutants

(e.g., particulate matter [PM], O_3, lead [Pb], environmental tobacco smoke [ETS], radiation) do not exhibit thresholds for cancer or noncancer health effects, even though likely mechanisms include nonlinear processes for some key events.

Finally, identification of the population groups or lifestages that may be at greater risk of health effects from air pollutant exposures contributes to an understanding of the public health impact of pollutant exposures. In the ISA, the term "at-risk population" is used to encompass populations or lifestages that have a greater likelihood of experiencing health effects related to exposure to an air pollutant due to a variety of factors; other terms used in the literature include susceptible, vulnerable, and sensitive. These factors may be intrinsic, such as genetic or developmental factors, race, gender, lifestage, or the presence of preexisting diseases, or they may be extrinsic, such as socioeconomic status (SES), activity pattern and exercise level, reduced access to health care, low educational attainment, or increased pollutant exposures (e.g., near roadways). Epidemiologic studies can help identify populations potentially at increased risk of effects by evaluating health responses in the study population. Examples include testing for interactions or effect modification by factors such as gender, age group, or health status. Experimental studies using animal models of susceptibility or disease can also inform the extent to which health risks are likely greater in specific population groups.

Quantitative Relationships: Effects on Ecosystems or Public Welfare

Key questions for understanding the quantitative relationships between exposure (or concentration or deposition) to a pollutant and risk to ecosystems or the public welfare include:

- What elements of the ecosystem (e.g., types, regions, taxonomic groups, populations, functions, etc.) appear to be affected, or are more sensitive to effects? Are there differences between locations or materials in welfare effects responses, such as impaired visibility or materials damage?
- Under what exposure conditions (amount deposited or concentration, duration and pattern) are effects seen?
- What is the shape of the concentration-response or exposure-response relationship?

Evaluations of causality generally consider the probability of quantitative changes in ecological and welfare effects in response to exposure. A challenge to the quantification of exposure-response relationships for ecological effects is the great regional and local spatial variability, as well as temporal variability, in ecosystems. Thus, exposure-response relationships are often determined for a specific ecological system and scale,

rather than at the national or even regional scale. Quantitative relationships therefore are estimated site by site and may differ greatly between ecosystems.

Concepts in Evaluating Adversity of Health Effects

In evaluating health evidence, a number of factors can be considered in delineating between adverse and nonadverse health effects resulting from exposure to air pollution. Some health outcomes, such as hospitalization for respiratory or cardiovascular diseases, are clearly considered adverse. It is more difficult to determine the extent of change that constitutes adversity in more subtle health measures. These include a wide variety of responses, such as alterations in markers of inflammation or oxidative stress, changes in pulmonary function or heart rate variability, or alterations in neurocognitive function measures. The challenge is determining the magnitude of change in these measures when there is no clear point at which a change becomes adverse. The extent to which a change in health measure constitutes an adverse health effect may vary between populations. Some changes that may not be considered adverse in healthy individuals would be potentially adverse in more at-risk individuals.

The extent to which changes in lung function are adverse has been discussed by the American Thoracic Society (ATS) in an official statement titled *What Constitutes an Adverse Health Effect of Air Pollution?* (ATS, 2000). An air pollution-induced shift in the population distribution of a given risk factor for a health outcome was viewed as adverse, even though it may not increase the risk of any one individual to an unacceptable level. For example, a population of asthmatics could have a distribution of lung function such that no identifiable individual has a level associated with significant impairment. Exposure to air pollution could shift the distribution such that no identifiable individual experiences clinically relevant effects. This shift toward decreased lung function, however, would be considered adverse because individuals within the population would have diminished reserve function and therefore would be at increased risk to further environmental insult. The committee also observed that elevations of biomarkers, such as cell number and types, cytokines and reactive oxygen species, may signal risk for ongoing injury and clinical effects or may simply indicate transient responses that can provide insights into mechanisms of injury, thus illustrating the lack of clear boundaries that separate adverse from nonadverse effects.

The more subtle health outcomes may be connected mechanistically to health events that are clearly adverse. For example, air pollution may affect markers of transient myocardial ischemia such as ST-segment abnormalities or onset of exertional angina. These effects may not be apparent to the individual, yet may still increase the risk of a number of cardiac events, including myocardial infarction and sudden death. Thus, small changes in

physiological measures may not appear to be clearly adverse when considered alone, but may be a part of a coherent and biologically plausible chain of related health outcomes that range up to responses that are very clearly adverse, such as hospitalization or mortality.

Concepts in Evaluating Adversity of Ecological Effects

Adversity of ecological effects can be understood in terms ranging in biological level of organization; from the cellular level to the individual organism and to the population, community, and ecosystem levels. In the context of ecology, a population is a group of individuals of the same species, and a community is an assemblage of populations of different species interacting with one another that inhabit an area. An ecosystem is the interactive system formed from all living organisms and their abiotic (physical and chemical) environment within a given area (IPCC, 2007). The boundaries of what could be called an ecosystem are somewhat arbitrary, depending on the focus of interest or study. Thus, the extent of an ecosystem may range from very small spatial scales to, ultimately, the entire Earth (IPCC, 2007).

Effects on an individual organism are generally not considered to be adverse to public welfare. However if effects occur to enough individuals within a population, then communities and ecosystems may be disrupted. Changes to populations, communities, and ecosystems can in turn result in an alteration of ecosystem processes. Ecosystem processes are defined as the metabolic functions of ecosystems including energy flow, elemental cycling, and the production, consumption and decomposition of organic matter (U.S. EPA, 2002a). Growth, reproduction, and mortality are species-level endpoints that can be clearly linked to community and ecosystem effects and are considered to be adverse when negatively affected. Other endpoints such as changes in behavior and physiological stress can decrease ecological fitness of an organism, but are harder to link unequivocally to effects at the population, community, and ecosystem level. The degree to which pollutant exposure is considered adverse may also depend on the location and its intended use (i.e., city park, commercial, and cropland). Support for consideration of adversity beyond the species level by making explicit the linkages between stress-related effects at the species and effects at the ecosystem level is found in *A Framework for Assessing and Reporting on Ecological Condition: an SAB report* (U.S. EPA, 2002a). Additionally, the National Acid Precipitation Assessment Program (NAPAP, 1991) uses the following working definition of “adverse ecological effects” in the preparation of reports to Congress mandated by the Clean Air Act: “any injury (i.e., loss of chemical or physical quality or viability) to any ecological or ecosystem component, up to and including at the regional level, over both long and short terms.”

On a broader scale, ecosystem services may provide indicators for ecological impacts. Ecosystem services are the benefits that people obtain from ecosystems (UNEP, 2003). According to the Millennium Ecosystem Assessment, ecosystem services include: "provisioning services such as food and water; regulating services such as regulation of floods, drought, land degradation, and disease; supporting services such as soil formation and nutrient cycling; and cultural services such as recreational, spiritual, religious, and other nonmaterial benefits." For example, a more subtle ecological effect of pollution exposure may result in a clearly adverse impact on ecosystem services if it results in a population decline in a species that is recreationally or culturally important.

References for Preamble

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Legislative and Historical Background

Legislative Requirements for the NAAQS Review

Two sections of the Clean Air Act (CAA) govern the establishment and revision of the NAAQS. Section 108 (42:U.S.C.:7408) directs the Administrator to identify and list certain air pollutants and then to issue air quality criteria for those pollutants. The Administrator is to list those air pollutants that in her "… judgment, cause or contribute to air pollution which may reasonably be anticipated to endanger public health or welfare; …" and, "… the presence of which in the ambient air results from numerous or diverse mobile or stationary sources;" and, "… for which … [the Administrator] plans to issue air quality criteria…." Air quality criteria are intended to "accurately reflect the latest scientific knowledge useful in indicating the kind and extent of all identifiable effects on public health or welfare which may be expected from the presence of [a] pollutant in the ambient air …" (42:U.S.C.:7408([b]). Section 109 (42:U.S.C.:7409) directs the Administrator to propose and promulgate "primary" and "secondary" NAAQS for pollutants for which air quality criteria are issued. Section 109(b)(1) defines a primary standard as one "…the attainment and maintenance of which in the judgment of the Administrator, based on such criteria and allowing an adequate margin of safety, are requisite to protect the public health." The legislative history of Section 109 indicates that a primary standard is to be set at "… the maximum permissible ambient air level … which will protect the health of any [sensitive] group of the population," and that for this purpose "… reference should be made to a representative sample of persons comprising the sensitive group rather than to a single person in such a group…" (S. Rep. No. 91:1196, 91st Cong., 2d Sess. 10 [1970]). A secondary standard, as defined in Section 109(b)(2), must "… specify a level of air quality the attainment and maintenance of which, in the judgment of the Administrator, based on such criteria, is requisite to protect the public welfare from any known or anticipated adverse effects associated with the presence of [the] pollutant in the ambient air." Welfare effects (as defined in Section 302(h); 42:U.S.C.:7602[h]) include, but are not limited to, "… effects on soils, water, crops, vegetation, man-made materials, animals, wildlife, weather, visibility and climate, damage to and deterioration of property, and hazards to transportation, as well as effects on economic values and on personal comfort and well-being."

The requirement that primary standards provide an adequate margin of safety was intended to address uncertainties associated with inconclusive scientific and technical information available at the time of standard setting. It was also intended to provide a reasonable degree of protection against hazards that research has not yet identified (*Lead Industries Association v. EPA,* 647:F.2d:1130-1154 [D.C.Cir 1980]; *American Petroleum*

Institute v. Costle, 665:F.2d:1176-1186 [D.C.Cir. 1981]; *American Farm Bureau Federation v. EPA*, 559:F.3d:512-533 [D.C. Cir. 2009]; *Association of Battery Recyclers v. EPA*, 604:F.3d:613, 617-618 [D.C. Cir. 2010]). Both kinds of uncertainties are components of the risk associated with pollution at levels below those at which human health effects can be said to occur with reasonable scientific certainty. Thus, in selecting primary standards that provide an adequate margin of safety, the Administrator is seeking not only to prevent pollution levels that have been demonstrated to be harmful but also to prevent lower pollutant levels that may pose an unacceptable risk of harm, even if the risk is not precisely identified as to nature or degree. The CAA does not require the Administrator to establish a primary NAAQS at a zero-risk level or at background concentration levels (*Lead Industries v. EPA*, [647:F.2d:at 1156 n.51]), but rather at a level that reduces risk sufficiently so as to protect public health with an adequate margin of safety.

In addressing the requirement for an adequate margin of safety, the EPA considers such factors as the nature and severity of the health effects involved, the size of sensitive population(s) at risk, and the kind and degree of the uncertainties that must be addressed. The selection of any particular approach to providing an adequate margin of safety is a policy choice left specifically to the Administrator's judgment (*Lead Industries Association v. EPA*, [647:F.2d:1161-1162]; *Whitman v. American Trucking Associations*, [531:U.S.:457-495 (2001)]).

In setting standards that are "requisite" to protect public health and welfare as provided in Section 109(b), EPA's task is to establish standards that are neither more nor less stringent than necessary for these purposes. In so doing, EPA may not consider the costs of implementing the standards (see generally, *Whitman v. American Trucking Associations*, [531:U.S.:457, 465-472, 475-476 (2001)]). Likewise, "… [a]ttainability and technological feasibility are not relevant considerations in the promulgation of national ambient air quality standards." (*American Petroleum Institute v. Costle*, [665:F.2d:1185]).

Section 109(d)(1) requires that "not later than December 31, 1980, and at 5-year intervals thereafter, the Administrator shall complete a thorough review of the criteria published under Section 108 and the national ambient air quality standards … and shall make such revisions in such criteria and standards and promulgate such new standards as may be appropriate … ." Section 109(d)(2) requires that an independent scientific review committee "shall complete a review of the criteria … and the national primary and secondary ambient air quality standards … and shall recommend to the Administrator any new … standards and revisions of existing criteria and standards as may be appropriate … ." Since the early 1980's, this independent review function has been performed by the Clean Air Scientific Advisory Committee (CASAC).

History of the NAAQS for Pb

Unlike pollutants such as PM and carbon monoxide (CO), air quality criteria had not been issued for Pb as of the enactment of the Clean Air Act of 1970, which first set forth the requirement to set national ambient air quality standards for criteria pollutants. EPA did not intend to issue air quality criteria for lead, and accordingly had not listed lead under Section 108. EPA had determined to control lead air pollution through regulations to phase-out use of lead additives in gasoline and EPA viewed those controls, and possibly additional federal controls, as the best approach to controlling lead emissions (See 41 FR 14921 (April 8, 1976). However, the decision not to list lead under Section 108 was challenged by environmental and public health groups and the U.S. District Court for the Southern District of New York concluded that EPA was required to list lead under Section 108. (*Natural Resources Defense Council v. EPA*, 411 F. Supp. 864 [S.D. N.Y. 1976], aff'd, 545 F.2d 320 [2d Cir. 1978]).

Accordingly, on April 8, 1976, EPA published a notice that Pb had been listed under Section 108 as a criteria pollutant (41 FR 14921) and on October 5, 1978, EPA promulgated primary and secondary NAAQS for Pb under Section 109 of the Act (43 FR 46246). Both primary and secondary standards were set at a level of 1.5 micrograms per cubic meter ($\mu g/m^3$), measured as Pb in total suspended particles (Pb-TSP), not to be exceeded by the maximum arithmetic mean concentration averaged over a calendar quarter. These standards were based on the 1977 Pb Air Quality Criteria for Lead Document (AQCD) (U.S. EPA, 1977).

The first review of the Pb standards was initiated in the mid-1980s. The scientific assessment for that review is described in the 1986 Pb AQCD (U.S. EPA, 1986a), the associated Addendum (U.S. EPA, 1986c) and the 1990 Supplement (U.S. EPA, 1990a). As part of the review, the Agency designed and performed human exposure and health risk analyses (U.S. EPA, 1989), the results of which were presented in a 1990 Staff Paper

(U.S. EPA, 1990c). Based on the scientific assessment and the human exposure and health risk analyses, the 1990 Staff Paper presented recommendations for consideration by the Administrator (U.S. EPA, 1990c). After consideration of the documents developed during the review and the significantly changed circumstances since Pb was listed in 1976, the Agency did not propose any revisions to the 1978 Pb NAAQS. In a parallel effort, the Agency developed the broad, multi-program, multimedia, integrated U.S. Strategy for Reducing Lead Exposure (U.S. EPA, 1991). As part of implementing this strategy, the Agency focused efforts primarily on regulatory and remedial clean-up actions aimed at reducing Pb exposures from a variety of non-air sources judged to pose more extensive public health risks to U.S. populations, as well as on actions to reduce Pb emissions to air, such as bringing more areas into compliance with the existing Pb NAAQS (U.S. EPA, 1991).

The most recent review of the Pb air quality criteria and standards was initiated in November, 2004 (69 FR 64926) and the Agency's plans for preparation of the *Air Quality Criteria Document* (AQCD) and conduct of the NAAQS review were contained in two documents: *Project Work Plan for Revised Air Quality Criteria for Lead* (U.S. EPA, 2005e); and *Plan for Review of the National Ambient Air Quality Standards for Lead* (U.S. EPA, 2006f). The schedule for completion of this review was governed by a judicial order in *Missouri Coalition for the Environment v. EPA* (No. 4:04CV00660 ERW, Sept. 14, 2005; and amended on April 29, 2008 and July 1, 2008), which specified a schedule for the review of duration substantially shorter than five years.

The scientific assessment for the review is described in the 2006 *Air Quality Criteria for Lead* [2006 Pb AQCD; (U.S. EPA, 2006b)], multiple drafts of which received review by CASAC and the public. EPA also conducted human exposure and health risk assessments and a pilot ecological risk assessment for the review, after consultation with CASAC and receiving public comment on a draft analysis plan (U.S. EPA, 2006d). Drafts of these quantitative assessments were reviewed by CASAC and the public. The pilot ecological risk assessment was released in December 2006 (ICF, 2006) and the final health risk assessment report was released in November 2007 (U.S. EPA, 2007g). The policy assessment based on both of these assessments, air quality analyses and key evidence from the AQCD was presented in the Staff Paper (U.S. EPA, 2006g), a draft of which also received CASAC and public review. The final Staff Paper presented OAQPS staff's evaluation of the public health and welfare policy implications of the key studies and scientific information contained in the 2006 Pb AQCD and presented and interpreted results from the quantitative risk/exposure analyses conducted for this review. Based on this evaluation, the Staff Paper presented OAQPS staff recommendations that the Administrator give consideration to substantially revising the primary and secondary standards to a range of levels at or below 0.2 $\mu g/m^3$.

Immediately subsequent to completion of the Staff Paper, EPA issued an advance notice of proposed rulemaking (ANPR) that was signed by the Administrator on December 5, 2007 (72 FR 71488).[1] CASAC provided advice and recommendations to the Administrator with regard to the Pb NAAQS based on its review of the ANPR and the previously released final Staff Paper and risk assessment reports. The proposed decision on revisions to the Pb NAAQS was signed on May 1, 2008 and published in the Federal Register on May 20, 2008 (73 FR 29184). Members of the public provided both written and, at two public hearings, oral comments and the CASAC Pb Panel also provided advice and recommendations to the Administrator based on its review of the proposal notice. The final decision on revisions to the Pb NAAQS was signed on October 15, 2008 and published in the Federal Register on November 12, 2008 (73 FR 66964).

The November 2008 notice described EPA's decision to revise the primary and secondary NAAQS for Pb from a level of 1.5 $\mu g/m^3$ to a level of 0.15 $\mu g/m^3$. EPA's decision on the level for the primary standard was based on the much-expanded health effects evidence on neurocognitive effects of Pb in children. The level of 0.15 $\mu g/m^3$ was established to protect against air Pb-related health effects, including intelligence quotient (IQ) decrements in the most highly exposed children, those exposed at the level of the standard. Results of the quantitative risk assessment were judged supportive of the evidence-based framework estimates. The averaging time was revised to a rolling three-month period with a maximum (not-to-be-exceeded) form, evaluated over a three-year period. As compared to the previous averaging time of calendar quarter, this revision was considered to be more scientifically appropriate and more health protective. The rolling average gives equal weight to all three-month periods, and the new calculation method gives equal weight to each month within each three-month period. Further, the rolling average yields 12 three-month averages each year to be compared to the NAAQS versus four averages in each year for the block calendar quarters pertaining to the previous standard. The indicator of Pb-TSP was retained, reflecting the evidence that Pb particles of all sizes pose health risks. The secondary standard was revised to be identical in all respects to the revised primary standards.[2]

Revisions to the NAAQS were accompanied by revisions to the data handling procedures, the treatment of exceptional events, and the ambient air monitoring and

[1] The ANPR was one of the features of the revised NAAQS review process that EPA instituted in 2006. In 2009, this component of the process was replaced by reinstatement of the OAQPS policy assessment (previously termed the Staff Paper).
[2] The 2008 NAAQS for Pb are specified at 40 CFR 50.16.

reporting requirements, as well as emissions inventory reporting requirements.[1] One aspect of the new data handling requirements is the allowance for the use of Pb-PM_{10} monitoring for Pb NAAQS attainment purposes in certain limited circumstances at non-source-oriented sites. The monitoring network requirements resulted in a substantial number of new monitors being required as of January 2010, Subsequent to the 2008 rulemaking, additional revisions were made to the monitoring network requirements, which required additional monitors as of December 2011; the complete current requirements are described in Section 2.4.

On February 26, 2010 (75 FR 8934), EPA formally initiated its current review of the air quality criteria for Pb, requesting the submission of recent scientific information on specified topics. Soon after, a science policy workshop was held to identify key policy issues and questions to frame the review of the Pb NAAQS (75 FR 20843). Drawing from the workshop discussions, a draft IRP [*Integrated Review Plan for the National Ambient Air Quality Standards for Lead* (U.S. EPA, 2011d)], was developed and made available in late March, 2011 for public comment and consultation with CASAC and was discussed by the CASAC via a publicly accessible teleconference consultation on May 5, 2011 (76 FR 20347, 76 FR 21346). The final IRP (U.S. EPA, 2011c) was released in November, 2011 (76 FR 76972).

As part of the science assessment phase of the current review, EPA held a workshop in December 2010 (75 FR 69078) to discuss, with invited scientific experts, preliminary draft materials prepared during the ongoing development of the Pb ISA. The first external review draft *ISA for Lead* was released on May 6, 2011 (U.S. EPA, 2011e). The CASAC Pb Review Panel met at a public meeting on July 20, 2011 to review the draft ISA (76 FR 36120). Subsequently, on December 9, 2011, the CASAC panel provided a consensus letter for their review to the Administrator of the EPA (Frey and Samet, 2011). The second external review draft *ISA for Lead*, (U.S. EPA, 2012a) was discussed at a public meeting of the CASAC Pb Review Panel on April 10, 2012 (77 FR 14783). Subsequently, the CASAC panel provided a consensus letter for their review to the Administrator of the EPA(Frey, 2012). The third external review draft ISA for Lead was released on November 27, 2012(U.S. EPA, 2012b). The CASAC panel met at a public meeting on February 5, 2013, to review the draft ISA (78 FR 938). Subsequently, on June 4, 2013, the CASAC provided a consensus letter for their review to the Administrator of the EPA (Frey, 2013).

[1] The 2008 federal regulatory measurement methods for Pb are specified in 40 CFR 50, Appendix G and 40 CFR part 53. Consideration of ambient air measurements with regard to judging attainment of the standards is specified in 40 CFR 50, Appendix R. The Pb monitoring network requirements are specified in 40 CFR 58, Appendix D, Section 4.5. Guidance on the approach for implementation of the new standards was described in the Federal Register notices for the proposed and final rules (73 FR 29184; 73 FR 66964).

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Executive Summary

Introduction

This Integrated Science Assessment (ISA) is a synthesis and evaluation of the most policy-relevant science that forms the scientific foundation for the review of the primary (health-based) and secondary (welfare-based)[1] national ambient air quality standard (NAAQS) for Lead (Pb). In 2008, the levels of the primary and secondary NAAQS for Pb were lowered ten-fold, from the 1978 level of 1.5 $\mu g/m^3$, to a level of 0.15 $\mu g/m^3$. The averaging time was revised to a rolling three-month period with a maximum (not-to-be-exceeded) form, evaluated over a three-year period. EPA's decision on the level for the revised primary standard in 2008 was based on the substantially expanded body of health effects evidence available at that time, including that on cognitive effects of Pb in children. The revised standard was established to protect against air Pb-related human health effects, including intelligence quotient (IQ) loss, in the most highly exposed children.

The Preamble discusses the general framework for conducting the science assessment and developing an ISA, including criteria for selecting studies for inclusion in the ISA, evaluating and integrating the scientific evidence and developing scientific conclusions regarding the causal association of air pollution with health and environmental effects.

As described in Table II of the Preamble, the ISA uses a five-level hierarchy that classifies the weight of evidence for causation:

- Causal relationship
- Likely to be a causal relationship
- Suggestive of a causal relationship
- Inadequate to infer a causal relationship
- Not likely to be a causal relationship

Studies most relevant to the review of the NAAQS are highlighted in the ISA. In drawing judgments regarding causality for the criteria air pollutants, evidence of health and environmental effects in the range of relevant pollutant exposures or biomarker concentrations is considered. Considerations that are specific to the causal determinations drawn for the health and ecological effects of Pb are described in more detail in

[1] Welfare effects as defined in Clean Air Act (CAA) Section 302(h) [42 U.S.C. 7602(h)] include, but are not limited to, "effects on soils, water, crops, vegetation, man-made materials, animals, wildlife, weather, visibility and climate, damage to and deterioration of property, and hazards to transportation, as well as effects on economic values and on personal comfort and well-being."

Section 1.1. Briefly, both long and short-term Pb exposures were considered. Evidence from experimental animal studies observing effects of exposures resulting in blood Pb levels within an order of magnitude of those currently experienced by the U.S. general population was emphasized. With regard to the epidemiologic evidence, population-based studies using Pb biomarkers (i.e., blood or bone Pb concentrations) were emphasized over occupational studies. Relevant concentrations for drawing causality judgments for the welfare effects of Pb were determined considering Pb concentrations generally within one or two orders of magnitude above those which have been observed in the environment and the available evidence for concentrations at which effects were observed in plants, invertebrates, and vertebrates. Once conclusions are drawn, evidence relevant to quantifying the health or environmental risks is assessed.

Sources, Fate and Transport of Lead in the Environment, and the Resulting Human Exposure and Dose

Emissions of Pb to ambient air declined by more than two orders of magnitude over the period 1970 to 2008 following the ban on alkyl-Pb additives for on-road gasoline and tightened industrial emission standards. Air emissions in the U.S. were estimated to be 950 tons in 2008, a small fraction of the Pb processed in U.S. Pb-related industries. More than half of these emissions were from piston-engine aircraft. Other important sources of ambient air Pb, beginning with the next largest, include metals processing, fossil fuel combustion, other industrial sources, and roadway-related sources.

During the same period that saw the dramatic decrease in Pb emissions, ambient air Pb concentrations[1] also declined. The median value (across monitoring sites) for the maximum 3-month average concentration in 2010, 0.03 micrograms per cubic meter ($\mu g/m^3$), was approximately thirty times lower than it was in 1980. The sharpest drop in Pb concentration occurred from 1980-1990; concentrations continued to decline up to 2010. Specific levels near Pb sources as well as away from Pb sources have also shown a sharp decrease (Section 1.2.2).

The history of atmospheric deposition has led to measurable Pb concentrations in rain, snowpack, soil, surface waters, sediments, agricultural plants, livestock, and wildlife across the world, with the highest concentrations near Pb sources, such as smelters. After

[1] The original indicator for the Pb NAAQS is the mass of the Pb portion of total suspended particles (Pb-TSP). The Pb-TSP indicator was retained in 2008 in recognition of the role of all particulate matter (PM) sizes in ambient air Pb exposures (Section 1.2.2). The Federal Reference Method (FRM) Pb-TSP sampler's size-selective performance is known to be affected by wind speed and direction, and collection efficiency has been demonstrated to decline with particle size. Under certain conditions regulatory Pb monitoring can also be performed for ambient Pb sampled using the FRM for Pb sampled in particles with an upper 50% cut-point of 10 ± 0.5 micrometer (μm) aerodynamic diameter (Pb-PM_{10}). Pb-PM_{10} is allowed in certain instances where the expected Pb concentration does not approach the NAAQS and no sources of ultracoarse Pb particles are nearby.

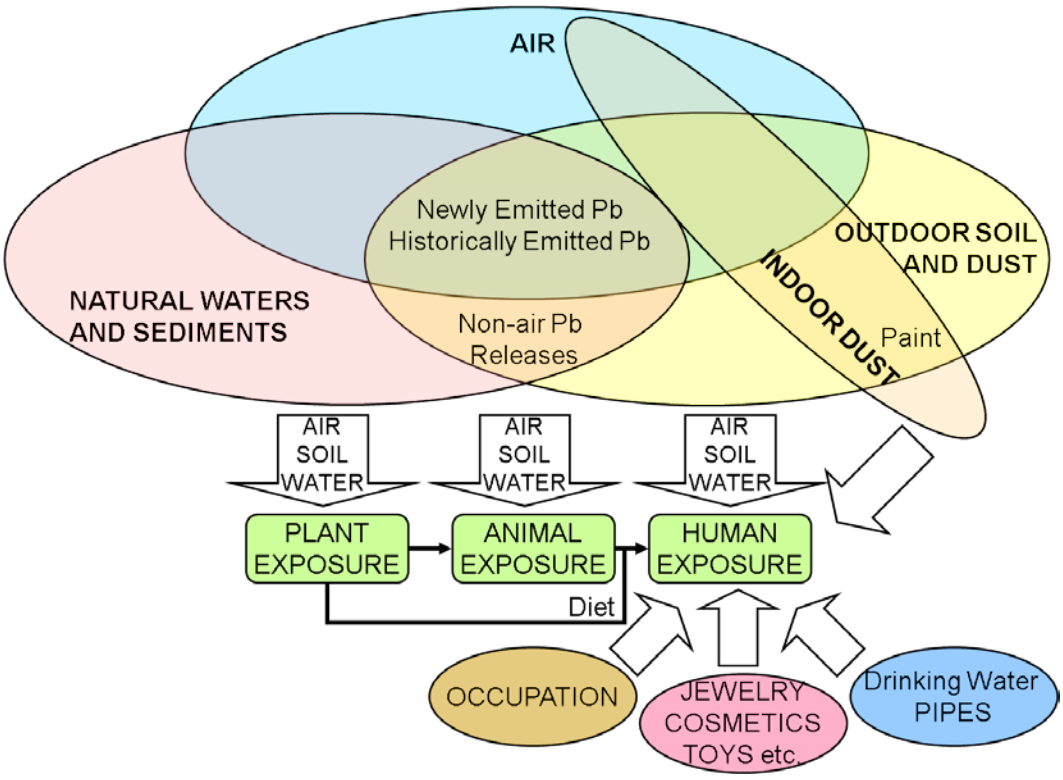
the phase-out of Pb from on-road gasoline and declining industrial emissions in the U.S., Pb concentrations have decreased considerably in rain, snowpack, and surface waters. Pb is retained in soils and sediments, where it provides a historical record of deposition and associated concentrations. The national average Pb concentration in soil in non-urban locations was 18.9 milligrams of Pb per kilogram (mg Pb/kg), measured in over 1,300 non-urban, generally vegetated sampling locations from 1961 through 1997. The national median fresh surface water dissolved Pb concentration (1991-2003) was 0.5 micrograms per liter (µg/L) (Section 1.2.3). In remote lakes, sediment profiles indicate higher Pb concentrations in near surface sediment as compared to pre-industrial era sediment from greater depth; sediment profiles indicate peak Pb concentrations between 1960 and 1980 (when industrial and mobile source Pb emissions in the U.S. were at their peak).

The size distribution of Pb-bearing particulate matter (PM), (i.e., PM having Pb as one of its components) depends on whether there are contributions from industrial sources or near-road environments (Section 1.2.2). Airborne particles containing Pb (Pb bearing PM[1]) tend to be small (much of the distribution <10 µm) compared with soil or dust particles containing Pb (~50 µm to several hundred µm). Coarse Pb-bearing PM (i.e., approximately 2.5 – 10 µm) deposits to a great extent near its source, contributing to local soil Pb contamination, while fine Pb-bearing PM (i.e., smaller than approximately 2.5 µm) can be transported long distances and possibly deposit in remote areas. Depending on local conditions, deposited particles may be resuspended and redeposited multiple times before further transport becomes unlikely.

There are multiple sources of Pb in the environment, and human exposure to Pb involves multiple pathways. Figure ES-1 shows how Pb can move through multiple environmental media. The “air/soil/water” arrows of the figure depict Pb exposures to plants, animals, and/or humans through contact with Pb-containing media. Air-related pathways of ambient Pb exposure are the focus of this assessment. Pathways of exposure to Pb from ambient air include both inhalation of Pb and ingestion of Pb in dust or soil that originated in the ambient air. For example, dietary Pb exposure may be air-related if ambient air Pb deposits on plant materials or in water that becomes available for human consumption. Dust and soil particles containing Pb are typically in the size range that is ingested rather than inhaled. However, soil can act as a reservoir for deposited Pb emissions, and exposure to soil contaminated with deposited Pb can occur through resuspended PM as well as hand-to-mouth contact, which is the main pathway of childhood exposure to Pb. The primary contribution of ambient air Pb to young children’s blood Pb concentrations is generally due to ingestion of Pb following its deposition in soils and dusts rather than inhalation of ambient air (Section 3.1.1.2). Non-ambient, non-

[1] Pb-bearing PM larger than 10 µm have a sharp concentration gradient with distance from the source, because larger particles have greater settling velocities.

air-related exposures include hand-to-mouth contact with Pb-containing consumer goods, hand-to-mouth contact with dust or chips of peeling Pb-containing paint, or ingestion of Pb in drinking water conveyed through Pb pipes. As a result of the multitude of possible exposure pathways, the contribution from specific pathways (e.g., consumer products, diet, soil, ambient air) to blood Pb concentrations is situation specific.



Note: This Venn diagram illustrates the passage of Pb through multiple environmental media compartments through which plant, animal, and human exposures can occur.

Figure ES-1 Conceptual model of multimedia Pb exposure.

The majority of Pb in the body is stored in bone (roughly 90% in adults, 70% in children). Much of the remaining Pb is found in soft tissues; only about 1% of Pb is found in the blood. Pb in blood is primarily (~99%) bound to red blood cells [RBCs]). The small fraction of Pb in blood plasma (<1% of Pb in blood) may be the more biologically labile and toxicologically active fraction of the circulating Pb. Both Pb uptake to and elimination from soft tissues are much faster than they are in bone. Pb accumulates in bone regions undergoing the most active calcification at the time of

exposure. Pb in bone becomes distributed in trabecular (e.g., patella [knee cap]) and the more dense cortical bones (e.g., tibia [shin bone]).

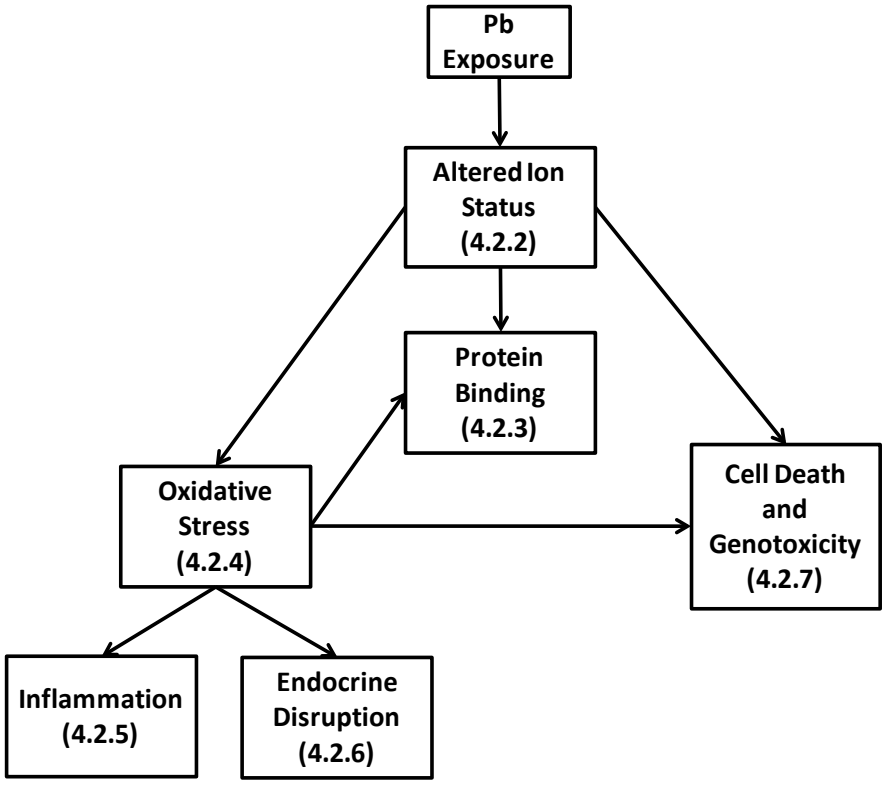
Blood Pb is the most common index of Pb exposure in epidemiologic studies of Pb health effects. Overall, blood Pb levels have been decreasing among U.S. children and adults for the past thirty-five years. The median blood Pb level for the U.S. population is 1.1 micrograms per deciliter (μg/dL), with a 95th percentile blood Pb level of 3.3 μg/dL based on the 2009–2010 National Health and Nutrition Examination Survey (NHANES) data. Among children aged 1-5 years, the median and 95th percentiles are slightly higher at 1.2 μg/dL and 3.4 μg/dL, respectively. Other common Pb exposure metrics used in epidemiologic studies are Pb in bone, which generally reflects cumulative exposure over long periods (months to years), and Pb in cord blood, which is an indicator of prenatal and neonatal blood Pb concentration.

Blood Pb is dependent on both the recent exposure history of the individual, as well as the long-term exposure history that determines total body burden and the amount of Pb stored in the bone. The contribution of bone Pb to blood Pb changes throughout an individual's life time, and depends on the duration and intensity of the exposure, age, and various other physiological stressors (e.g., nutritional status, pregnancy, menopause, extended bed rest, hyperparathyroidism) that may affect bone remodeling, which normally and continuously occurs. In children, largely due to faster exchange of Pb to and from bone, blood Pb is both an index of recent exposure and potentially an index of body burden. Generally, bone Pb is an index of cumulative exposure and body burden. Pb is sequestered in two types of bone compartments; Pb in trabecular bone exchanges more rapidly with the blood than Pb in denser cortical bone. Therefore, Pb in cortical bone is a better marker of cumulative exposure, and Pb in trabecular bone is more likely to be correlated with blood Pb concentration. During pregnancy, Pb is transferred from the mother to the fetus. Transplacental transfer of Pb may be facilitated by an increase in the plasma/blood Pb concentration ratio during pregnancy. Maternal-to-fetal transfer of Pb appears to be related partly to the mobilization of Pb from the maternal skeleton.

Integrative Overview of Health and Ecological Effects

There is substantial overlap between the ecological and human health endpoints related to Pb exposure, which can be mediated through multiple, interconnected modes of action (MOAs). The cellular/subcellular effect constituting the principal MOA for human health and ecological endpoints is altered ion status. Other related MOAs include protein binding, oxidative stress, inflammation, endocrine disruption, and cell death and genotoxicity (Figure ES-2). Since the mechanisms of Pb toxicity in some organ systems are the same or similar across species, many of the downstream health and ecological

effects are similar across species from invertebrates to vertebrates, including humans (Section 1.8.1).



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

Figure ES-2 Schematic representation of the relationships between the various MOAs by which Pb exerts its effects.

Health Effects of Pb

Evidence from epidemiologic and toxicological studies was considered in combination with the evidence from other disciplines such as toxicokinetics in determining the causal relationships for the health endpoints discussed in this assessment. Detailed discussions of the evidence relating to conclusions regarding the health effects of Pb are in Section 1.6 and Chapter 4. The major conclusions regarding health effects from Pb exposure in children and adults are presented in Table ES-1 and summarized below.

Table ES-1 Summary of causal determinations for the relationship between exposure to Pb and health effects.

Health Outcome	Causality Determination[a] (Table with Key Evidence)
Nervous System Effects (Section 1.6.1)	
Children – Nervous System Effects (Section 1.6.1.1)	
Cognitive Function Decrements	Causal Relationship (Table 4-17)
Clear evidence of cognitive function decrements (as measured by Full Scale IQ, academic performance, and executive function) in young children (4 to 11 years old) with mean or group blood Pb levels measured at various lifestages and time periods between 2 and 8 µg/dL. Clear support from animal toxicological studies that demonstrate decrements in learning, memory, and executive function with dietary exposures resulting in relevant blood Pb levels of 10-25 µg/dL. Plausible MOAs are demonstrated.	
Externalizing Behaviors: Attention, Impulsivity and Hyperactivity[b,d, e]	Causal Relationship (Table 4-17)
Clear evidence of attention decrements, impulsivity and hyperactivity (assessed using objective neuropsychological tests and parent and teacher ratings) in children 7-17 years and young adults ages 19-20 years. The strongest evidence for blood Pb-associated increases in these behaviors was found in prospective studies examining prenatal (maternal or cord), age 3-60 months, age 6 years, or lifetime average (to age 11-13 years) mean blood Pb levels of 7 to 14 µg/dL and groups with early childhood (age 30 months) blood Pb levels >10 µg/dL. Biological plausibility is provided by animal toxicological studies demonstrating impulsivity or impaired response inhibition with relevant prenatal, lactational, post-lactational and lifetime Pb exposures. Plausible MOAs are demonstrated.	
Externalizing Behaviors: Conduct Disorders in Children and Young Adults[c, d]	Likely Causal Relationship (Table 4-17)
Prospective epidemiologic studies find that early childhood (age 30 months, 6 years) or lifetime average (to age 11-13 years) blood Pb levels or tooth Pb levels (from ages 6-8 years) are associated with criminal offenses in young adults ages 19-24 years and with higher parent and teacher ratings of behaviors related to conduct disorders in children ages 8-17 years. Pb-associated increases in conduct disorders were found in populations with mean blood Pb levels 7 to14 µg/dL; associations with lower blood Pb levels as observed in cross-sectional studies were likely to be influenced by higher earlier Pb exposures. There is coherence in epidemiologic findings among related measures of conduct disorders. Evidence of Pb induced aggression in animals was mixed, with increases in aggression found in some studies of adult animals with gestational plus lifetime Pb exposure but not juvenile animals. The lack of clear biological plausibility produces some uncertainty.	
Internalizing Behaviors	Likely Causal Relationship (Table 4-17)
Prospective epidemiologic studies find associations of higher lifetime average blood (mean: ~14 µg/dL) or childhood tooth (from ages 6-8 years) Pb levels with higher parent and teacher ratings of internalizing behaviors such as symptoms of depression or anxiety, and withdrawn behavior in children ages 8-13 years. Consideration of potential confounding by parental caregiving was not consistent and findings from cross-sectional studies in populations ages 5 and 7 years with mean blood Pb levels of 5 µg/dL were mixed. Animal toxicological studies demonstrate depression-like behaviors and increases in emotionality with relevant lactational exposures. Plausible MOAs are demonstrated.	
Auditory Function Decrements	Likely Causal Relationship (Table 4-17)
A prospective epidemiologic study and large cross-sectional studies indicate associations between blood Pb levels and increased hearing thresholds at ages 4-19 years. Across studies, associations were found with blood Pb levels measured at various time periods, including prenatal maternal, neonatal (10 day, mean 4.8 µg/dL), lifetime average, and concurrent (ages 4-19 years) blood Pb levels (median 8 µg/dL). Plausible MOAs are demonstrated. The lack of biological plausibility in animals with relevant exposures produces some uncertainty.	

Table ES-1 (Continued): Summary of causal determinations for the relationship between exposure to Pb and health effects.

Health Outcome	Causality Determination[a] (Table with Key Evidence)
Visual Function Decrements	Inadequate to Infer a Causal Relationship (Table 4-17)
The available epidemiologic and toxicological evidence is of insufficient, quantity, quality and consistency.	
Motor Function Decrements	Likely Causal Relationship (Table 4-17)
Prospective epidemiologic studies provide evidence of associations of fine and gross motor function decrements in children ages 4-17 years with lifetime average blood Pb levels and with blood Pb levels measured at various time periods with means generally ranging from 4.8 to 12 µg/dL. Results were inconsistent in cross sectional studies with concurrent blood Pb level means 2-5 µg/dL. Limited evidence in animal toxicological studies with relevant Pb exposures.	
Adults – Nervous System Effects (Section 1.6.1.2)	
Cognitive Function Decrements	Likely Causal Relationship (Table 4-17)
Prospective studies indicate associations of higher baseline bone Pb levels with declines in cognitive function (executive function, visuospatial skills, learning and memory) in adults (>age 50 years) over 2- to 4-year periods. Cross-sectional studies provide additional support. Uncertainties remain regarding the timing, frequency, duration and level of the Pb exposures contributing to the effects observed and residual confounding by age. Biological plausibility is provided by findings that relevant lifetime Pb exposures from gestation, birth, or after weaning induce learning impairments in adult animals and by evidence demonstrating plausible MOAs.	
Psychopathological Effects	Likely Causal Relationship (Table 4-17)
Cross-sectional studies in a few populations demonstrate associations of higher concurrent blood or tibia Pb levels with self-reported symptoms of depression and anxiety in adults. Uncertainties remain regarding the timing, frequency, duration and level of Pb exposures contributing to the observed associations and residual confounding by age. Observations of depression-like behavior in animals with dietary lactational Pb exposure, with some evidence at relevant blood Pb levels, and evidence demonstrating plausible MOAs in experimental animals provides support.	
Auditory Function Decrements	Suggestive of a Causal Relationship (Table 4-17)
A high-quality prospective epidemiologic study finds associations of higher tibia Pb level with a greater rate of elevations in hearing threshold over 20 years. Some evidence indicates effects on relevant MOAs but important uncertainties remain related to effects on auditory function in animals with relevant Pb exposures.	
Visual Function Decrements	Inadequate to Infer a Causal Relationship (Table 4-17)
The available epidemiologic and toxicological evidence is of insufficient, quantity, quality and consistency.	
Neurodegenerative Diseases	Inadequate to Infer a Causal Relationship (Table 4-17)
The available epidemiologic and toxicological evidence is of insufficient, quantity, quality and consistency.	
Cardiovascular Effects (Section 1.6.2)	
Hypertension	Causal Relationship (Table 4-24)
Prospective epidemiologic studies with adjustment for multiple potential confounders consistently find associations of blood and bone Pb levels with hypertension incidence and increased blood pressure (BP) in adults. Cross-sectional studies provide supporting evidence. Meta-analyses underscore the consistency and reproducibility of the Pb associated increase in blood pressure and hypertension (a doubling of concurrent blood Pb level (between 1 and 40 µg/dL) is associated with a 1 mmHg increase in systolic BP); however, uncertainties remain regarding the timing, frequency, duration and level of Pb exposures contributing to the effects observed in epidemiologic studies. Experimental animal studies demonstrate effects on BP after long-term Pb exposure resulting in mean blood Pb levels of 10 µg/dL or greater. Plausible MOAs are demonstrated.	
Subclinical Atherosclerosis	Suggestive of a Causal Relationship (Table 4-24)
Cross-sectional analyses of NHANES data find associations of blood Pb level with peripheral artery disease (PAD) in adults. Animal toxicological evidence is limited to studies of MOA (oxidative stress, inflammation, endothelial cell dysfunction) that demonstrate biologically plausible mechanisms through which Pb exposure may initiate atherosclerotic vessel disease.	

Table ES-1 (Continued): Summary of causal determinations for the relationship between exposure to Pb and health effects.

Health Outcome	Causality Determination[a] (Table with Key Evidence)
Coronary Heart Disease	Causal Relationship (Table 4-24)
Prospective epidemiologic studies consistently find associations of Pb biomarkers with cardiovascular mortality and morbidity, specifically myocardial infarction (MI), ischemic heart disease (IHD), or HRV; however, uncertainties remain regarding the timing, frequency, duration and level of Pb exposures contributing to the effects observed in epidemiologic studies. Thrombus formation was observed in animals after relevant long term exposure and MOAs (hypertension, decreased HRV, increased corrected QT (QTc) interval, and corrected QRS complex (QRSc) duration in electrocardiogram [ECG]) are demonstrated in humans and animals.	
Cerebrovascular Disease	Inadequate to Infer a Causal Relationship (Table 4-24)
The available epidemiologic and toxicological evidence is of insufficient, quantity, quality, and/or consistency. Plausible MOAs, which are shared with hypertension and atherosclerosis, are demonstrated.	
Renal Effects (Section 1.6.3)	
Reduced Kidney Function	Suggestive of a Causal Relationship (Table 4-31)
Multiple high quality epidemiologic studies provide evidence that Pb exposure is associated with reduced kidney function; however, uncertainty remains regarding the potential for reverse causality to explain findings in humans. Further, inconsistencies and limitations in occupational studies, epidemiologic studies of children and clinical trials of chelation of CKD patient preclude strong inferences to be drawn based on their results. Although longitudinal studies found Pb-associated decrements in renal function in populations with mean blood Pb levels of 7 and 9 µg/dL, the contributions of higher past Pb exposures cannot be excluded. Animal toxicological studies demonstrate Pb-induced kidney dysfunction at blood Pb levels greater than 30 µg/dL; however, evidence in animals with blood Pb levels < 20 µg/dL is generally not available. At blood Pb levels between 20 and 30 µg/dL studies provide some evidence for dysfunction in kidney function measures (e.g., decreased creatinine clearance, increased serum creatinine, increased BUN). Plausible MOAs (Pb induced hypertension, renal oxidative stress and inflammation, morphological changes, and increased uric acid) are demonstrated.	
Immune System Effects (Section 1.6.4)	
Atopic and Inflammatory Responses	Likely Causal Relationship (Table 4-34)
Prospective studies of children ages 1-5 years indicate associations of prenatal cord and childhood blood Pb levels with asthma and allergy. This evidence is supported by cross-sectional associations between higher concurrent blood Pb levels (>10 µg/dL) in children and higher IgE. Uncertainties related to potential confounding by SES, smoking or allergen exposure are reduced through consideration of the evidence from experimental animal studies. The biological plausibility for the effects of Pb on IgE is provided by consistent findings in animals with gestational or gestational-lactational Pb exposures, with some evidence at blood Pb levels relevant to humans. Strong evidence of Pb-induced increases in Th2 cytokine production and inflammation in animals demonstrates MOA.	
Decreased Host Resistance	Likely Causal Relationship (Table 4-34)
Animal toxicological studies provide the majority of the evidence for Pb-induced decreased host resistance. Dietary Pb exposure producing relevant blood Pb levels (7-25 µg/dL) results in increased susceptibility to bacterial infection and suppressed delayed type hypersensitivity. Further, evidence demonstrating plausible MOA, including suppressed production of Th1 cytokines and decreased macrophage function in animals, provides coherence.	
Autoimmunity	Inadequate to Infer a Causal Relationship (Table 4-34)
The available toxicological and epidemiologic studies do not sufficiently inform Pb-induced generation of auto-antibodies with relevant Pb exposures.	

Table ES-1 (Continued): Summary of causal determinations for the relationship between exposure to Pb and health effects.

Health Outcome	Causality Determination[a] (Table with Key Evidence)
Hematologic Effects (Section 1.6.5)	
Decreased Red Blood Cell (RBC) Survival and Function	Causal Relationship (Table 4-35)
Animal toxicological studies demonstrate that exposures resulting in blood Pb levels relevant to humans (2-7 µg/dL) alter several hematological parameters (Hemoglobin [Hb], Hematocrit [Hct], and mean corpuscular volume [MCV]),increase measures of oxidative stress and increase cytotoxicity in red blood cell (RBC) precursor cells. Limited body of epidemiologic studies provides additional support for the association of Pb exposure with these endpoints. Plausible MOAs are demonstrated in experimental animals.	
Altered Heme Synthesis	Causal Relationship (Table 4-35)
Consistent findings from studies in experimental adult animal studies report that relevant exposures (e.g. blood Pb levels of 6.5 µg/dL) cause decreased ALAD and ferrochelatase activities. Additional support is garnered from a larger body of ecotoxicological studies demonstrating decreased ALAD activity across a wide range of species and a limited body of epidemiologic studies. Plausible MOAs are demonstrated in experimental animals.	
Reproductive and Developmental Effects (Section 1.6.6)	
Development	Causal Relationship (Table 4-48)
Multiple cross-sectional epidemiologic studies report associations between concurrent blood Pb levels and delayed pubertal onset for girls (6-18 years) and boys (8-15 years). These associations are consistently observed in populations with concurrent blood Pb levels 1.2-9.5 µg/dL. Few studies consider confounding by nutrition. Uncertainties remain regarding the timing, frequency, duration and level of Pb exposures contributing to the effects observed in epidemiologic studies of older children. Experimental animal studies demonstrate delayed onset of puberty in female pups with blood Pb levels of 1.3-13 µg/dL and delayed male sexual maturity at blood Pb levels of 34 µg/dL.	
Birth Outcomes (e.g., low birth weight, spontaneous abortion)	Suggestive of Causal Relationship (Table 4-48)
Some well-conducted epidemiologic studies report associations of maternal Pb biomarkers or cord blood Pb with preterm birth and low birth weight/fetal growth; however, the epidemiologic evidence is inconsistent overall and findings from experimental animal studies are mixed.	
Male Reproductive Function	Causal Relationship (Table 4-48)
Key evidence is provided by toxicological studies in rodents, non-human primates, and rabbits showing detrimental effects on semen quality, sperm and fecundity/fertility with supporting evidence in epidemiologic studies. Toxicological studies with relevant Pb exposure routes leading to blood Pb concentrations ranging from 5-43 µg/dL reported effects on sperm quality and sperm production rate, sperm DNA damage, and histological or ultrastructural damage to the male reproductive organs. Consistent associations in studies of occupational populations with concurrent blood Pb levels of 25 µg/dL and greater, report detrimental effects of Pb on sperm; however, uncertainties remain regarding the timing, frequency, duration and level of Pb exposures contributing to the effects observed in epidemiologic studies.	
Female Reproductive Function	Suggestive of Causal Relationship (Table 4-48)
Although findings are mixed overall, the body of evidence include some high-quality epidemiologic and toxicological studies, suggesting that Pb may affect some aspects of female reproductive function (hormone level, placental pathology).	

Table ES-1 (Continued): Summary of causal determinations for the relationship between exposure to Pb and health effects.

Health Outcome	Causality Determination[a] (Table with Key Evidence)
Cancer (Section 1.6.7)	
Cancer	Likely Causal Relationship (Table 4-50)
The animal toxicological literature provides the strong evidence for long-term exposure (i.e., 18 months or 2 years) to high concentrations of Pb (> 2,600 ppm) inducing tumor development; findings from epidemiologic studies inconsistent. Plausible MOAs are demonstrated.	

[a] In drawing conclusions regarding the causal relationship between Pb exposure and human health effects, evidence in the range of relevant pollutant exposures or biomarker levels was considered. Specifically, population-based epidemiology studies were emphasized with the recognition that many of the U.S populations studied included individuals with higher past than recent Pb exposures. Evidence from toxicological studies of effects observed in experimental animals at biomarker levels (e.g. blood Pb) comparable to those currently experienced by the U.S. general population were emphasized. Generally, studies with dietary exposures resulting in blood Pb levels within one order of magnitude above the upper end of the distribution of U.S. blood Pb levels were considered in forming concusions, with the majority of studies reporting blood Pb levels below 30 µg/dL. Studies with higher blood Pb levels were considered if they informed the evaluation of MOA, mechanisms, or kinetics. (Preamble, Section 1.1).

[b] Within the attention deficit hyperactivity disorder domain of externalizing behaviors, studies of Pb exposure have focused primarily on attention, impulsivity, and hyperactivity. Because the studies of ADHD were limited in terms of their design and did not adequately consider potential confounding by factors such as SES, parental education, or parental caregiving quality, they were not a major consideration in drawing conclusions about the relationship between Pb exposure and attention, impulsivity, and hyperactivity.

[c] Two domains of conduct disorders,(i.e., undersocialized aggressive conduct disorder and socialized aggressive conduct disorder), are combined for the purpose of this assessment because it is difficult to differentiate between these two domains in the available epidemiologic studies, which examine multiple endpoints such as delinquent behavior, aggression, antisocial behavior. Criminal offenses are included in the evaluation because they can be predicted by earlier conduct disorders (Section 4.3.3.2).

[d] There was limited evaluation of potential confounding by parental psychopathology, which is a strong risk factor for externalizing behaviors, in the majority of the epidemiologic studies; however, evidence of an association of between psychopathology in parents and Pb exposure in their children is not available (Section 4.3.3).

[e] Strong evidence from experimental animal studies reduces uncertainty related to confounding generally.

Effects of Pb Exposure in Children

Multiple epidemiologic studies conducted in diverse populations of children consistently demonstrate the harmful effects of Pb exposure on cognitive function (as measured by IQ decrements, decreased academic performance and poorer performance on tests of executive function). Blood Pb-associated effects on cognitive function were found in populations of children (ages 4-10) with mean or group blood Pb levels measured concurrently or earlier in the range of 2-8 µg/dL[1]. Evidence suggests that some Pb-related cognitive effects may be irreversible and that the neurodevelopmental effects of Pb exposure may persist into adulthood (Section 1.9.4). Epidemiologic studies also demonstrate that Pb exposure is associated with decreased attention, and increased impulsivity and hyperactivity in children (externalizing behaviors). This is supported by findings in animal studies demonstrating both analogous effects and biological plausibility at relevant exposure levels. Pb exposure can also exert harmful effects on blood cells and blood producing organs, and is likely to cause an increased risk of symptoms of depression and anxiety and withdrawn behavior (internalizing behaviors),

[1] The age range and blood Pb levels are based on studies described in detail in Section 4.3.2.

decreases in auditory and motor function, asthma and allergy, as well as conduct disorders in children and young adults. There is some uncertainty about the Pb exposures contributing to the effects and blood Pb levels observed in epidemiologic studies; however, these uncertainties are greater in studies of older children and adults than in studies of young children (Section 1.9.5). Despite these uncertainties, it is clear that Pb exposure in childhood presents a risk; further, there is no evidence of a threshold below which there are no harmful effects on cognition from Pb exposure.

Effects of Pb Exposure in Adults

A large body of evidence from both epidemiologic studies of adults and experimental studies in animals demonstrates the effect of long-term Pb exposure on increased blood pressure (BP) and hypertension (Section 1.6.2). In addition to its effect on BP, Pb exposure can also lead to coronary heart disease and death from cardiovascular causes and is associated with cognitive function decrements, symptoms of depression and anxiety, and immune effects in adult humans. The extent to which the effects of Pb on the cardiovascular system are reversible is not well-characterized. Additionally, the frequency, timing, level, and duration of Pb exposure causing the effects observed in adults has not been pinpointed, and higher past exposures may contribute to the development of health effects measured later in life. It is clear however, that Pb exposure can result in harm to the cardiovascular system that is evident in adulthood and may also affect a broad array of organ systems.

Ecological Effects of Pb

Ecological effects of Pb are summarized for terrestrial, freshwater and saltwater ecosystems, and the ISA discusses endpoints common to plants, invertebrates and vertebrates along with considerations of uncertainties in relating atmospheric Pb concentrations to ecosystem effects. Effects of Pb in ecosystems are primarily associated with Pb deposition onto soil and water, subsequent transport, and exposure through environmental media (soil, water, sediment, biota). The 2006 Pb Air Quality Criteria Document (AQCD) (U.S. EPA, 2006b) and previous EPA assessments reported effects of Pb exposure on both terrestrial and aquatic organisms that included reduced survival, reproduction and growth as well as effects on behavior, development, and heme production. Studies reviewed in this ISA generally support the ecological findings of previous Pb assessments with some effects observed in additional species and at lower concentrations. Reproduction, growth, and survival are endpoints commonly used in ecological risk assessment because they can lead to effects at the population, community,

and ecosystem levels of biological organization. Impacts on hematological, neurobehavioral and physiological stress endpoints may increase susceptibility to other stressors and affect the fitness of individual organisms. Increasing exposures generally result in increasing responses in laboratory and field experiments but the relationship of exposure and responses is difficult to characterize quantitatively in natural systems because of the influence of multiple environmental variables on both Pb bioavailability and toxicity, and substantial species and lifestage differences in Pb sensitivity.

A brief discussion of the conclusions from this assessment and earlier Pb AQCDs regarding Pb effects on reproduction, growth, and survival is provided below and summarized in Table ES-2 along with effects of Pb on neurobehavior, hematological, and stress endpoints. Causal determinations for ecological effects were based on integration of information on biogeochemistry, bioavailability, biological effects, and exposure-response relationships of Pb in terrestrial, freshwater, and saltwater environments. In general, the number of studies available for assessing causality is greater for freshwater organisms than for marine environments. A detailed discussion for all relevant welfare effects (i.e., ecological effects) is provided in Section 1.7 and Chapter 6.

Table ES-2 Summary of causal determinations for the relationship between Pb exposure and effect on plants, invertebrates, and vertebrates.

Level		Effect	Terrestrial[a]	Freshwater[a]	Saltwater[a]
Community- and Ecosystem		Community and Ecosystem Effects (Section 1.7.3.7)	Likely Causal	Likely Causal	Inadequate
Population–Level Endpoints	**Organism–Level Responses**	Reproductive and Developmental Effects-Plants (Section 1.7.3.1)	Inadequate	Inadequate	Inadequate
		Reproductive and Developmental Effects-Invertebrates (Section 1.7.3.1)	Causal	Causal	Suggestive
		Reproductive and Developmental Effects-Vertebrates (Section 1.7.3.1)	Causal	Causal	Inadequate
		Growth-Plants (Section 1.7.3.2)	Causal	Likely Causal	Inadequate
		Growth-Invertebrates (Section 1.7.3.2)	Likely Causal	Causal	Inadequate
		Growth-Vertebrates (Section 1.7.3.2)	Inadequate	Inadequate	Inadequate
		Survival-Plants (Section 1.7.3.3)	Inadequate	Inadequate	Inadequate
		Survival- Invertebrates (Section 1.7.3.3)	Causal	Causal	Inadequate
		Survival- Vertebrates (Section 1.7.3.3)	Likely Causal	Causal	Inadequate
		Neurobehavioral Effects-Invertebrates (Section 1.7.3.4)	Likely Causal	Likely Causal	Inadequate
		Neurobehavioral Effects-Vertebrates (Section 1.7.3.4)	Likely Causal	Likely Causal	Inadequate
	Sub-organismal Responses	Hematological Effects-Invertebrates (Section 1.7.3.5)	Inadequate	Likely Causal	Suggestive
		Hematological Effects-Vertebrates (Section 1.7.3.5)	Causal	Causal	Inadequate
		Physiological Stress-Plants (Section 1.7.3.6)	Causal	Likely Causal	Inadequate
		Physiological Stress-Invertebrates (Section 1.7.3.6)	Likely Causal	Likely Causal	Suggestive
		Physiological Stress-Vertebrates (Section 1.7.3.6)	Likely Causal	Likely Causal	Inadequate

[a]Conclusions are based on the weight of evidence for causal determination in Table II of the ISA Preamble. Ecological effects observed at or near ambient Pb concentrations measured in soil, sediment and water in the most recent available studies (Table 1-1). were emphasized and studies generally within one to two orders of magnitude above the reported range of these values were considered in the body of evidence for terrestrial (Section 6.3.12), freshwater (Section 6.4.12) and saltwater (Section 6.4.21) ecosystems.

Effects on Development and Reproduction

Reduced reproduction at the level of individual organisms can result in lowered population numbers or extermination, decreased species diversity, and a decline in relative or absolute population numbers at the community level. Effects of Pb on various development, fertility, and hormone maintenance endpoints have been documented in multiple species of terrestrial and freshwater organisms. In plants, only a few studies have addressed reproductive effects of Pb exposure. Among the animal species tested, freshwater invertebrates were the most sensitive to Pb with respect to reproduction (Section 1.7.3.1).

Effects on Growth

Effects on growth observed at the species level can translate into effects at the ecosystem level. Exposure to Pb has been shown to have effects on growth in plants and in some species of invertebrates and vertebrates. Evidence for effects of Pb on growth is strongest in terrestrial plants. These effects are typically found in laboratory studies with high Pb exposure concentrations or in field studies near stationary sources such as metal industries or mines where concentrations of multiple metals are elevated relative to non-polluted locations. Many of those laboratory and field studies evaluate the effects of increasing levels of Pb exposure, and find that effects on plant growth increase with increasing exposure ("biological gradients"). Evidence for Pb effects on growth in invertebrates has been observed most extensively in freshwater species, with growth inhibition in a few sensitive species occurring in the range of Pb concentration values available for U.S. surface waters. In general, juvenile organisms are more sensitive than adults. There are only limited data on growth effects in vertebrates (Section 1.7.3.2).

Effects on Survival

Decreased survival of individuals within a population can have ecosystem-level impacts. Pb is generally not toxic to aquatic or terrestrial plants at concentrations found in the environment away from stationary sources, probably due to the fact that plants often sequester large amounts of Pb in roots, with little translocation to other parts of the plant. Aquatic invertebrates are generally more sensitive to Pb exposure than other types of organisms, with survival reduced in laboratory studies of a few species at concentrations occurring near Pb sources, as well as at concentrations occasionally encountered in the general environment (that is, far from major Pb sources). Many terrestrial invertebrates tolerate higher concentrations of Pb. Limited studies with vertebrates showed adverse effects of Pb on survival at concentrations higher than typical ambient Pb levels in the

environment, although juvenile organisms are usually more sensitive than adults (Section 1.7.3.3).

Neurobehavioral Effects

Historical and recent evidence from Pb-exposed animals indicates that Pb affects behaviors, such as food consumption, avoidance and escape from predators, behavioral regulation of body temperature, and prey capture. Alterations to these behaviors can decrease the overall fitness of the organism. Evidence from laboratory studies has shown effects of Pb exposure on nervous system endpoints in both terrestrial and freshwater animal taxa (Section 1.7.3.4).

Hematological Effects

Changes in hematological characteristics including ALAD (delta-aminolevulinic acid dehydratase, an important rate-limiting enzyme needed for heme production) activity, blood cell counts, and serum profiles are associated with Pb exposure in both aquatic and terrestrial animals. It is commonly recognized that ALAD is an indicator of Pb exposure across a wide range of animals as shown in both field and laboratory studies. Studies conducted over the last two decades have shown that hematological responses are associated with Pb in the environment (Section 1.7.3.5).

Effects on Physiological Stress

Increased levels of antioxidant enzymes (in response to oxidative stress or altered cell signaling) and increased lipid peroxidation (the process by which free radicals induce the oxidation of fatty acids, leading to cell membrane damage) are considered to be reliable biomarkers of stress. Alterations in these biomarkers are associated with Pb exposure in plants, invertebrates and vertebrates, and they may be indicative of increased susceptibility to other stressors, as well as reduction in individual fitness. Markers of oxidative damage and antioxidant activity have been observed in field studies in a wide range of species in terrestrial and aquatic environments when Pb is present (along with other chemicals), and also following laboratory exposures (Section 1.7.3.6).

Community and Ecosystem Effects

The effects of Pb on growth, reproduction, and survival at the level of individual organisms, especially when considered cumulatively, are likely to result in effects on population, community and ecosystem structure and function. Effects at those higher

levels of biological organization are confirmed by both laboratory and field experiments. In these experiments decreases in abundance, reduced species diversity, shifts in soil microbial and plant community composition (in terrestrial ecosystems), and sediment-associated and aquatic plant community composition (in freshwater ecosystems) have been observed following Pb exposure. However, such ecosystem-wide effects can only be tested directly in a few of the cases where individual organism effects are found. Quantitative characterization of exposure-response relationships is difficult at the community and ecosystem levels because potential confounders such as the presence of other metals, physico-chemical variables and other stressors cannot be controlled and their effects are incompletely characterized (Section 1.7.3.7).

Policy Relevant Considerations

Public Health Significance

The 2006 Pb AQCD (U.S. EPA, 2006b) concluded that neurodevelopmental effects in children and cardiovascular effects in adults were of the greatest public health concern because the evidence indicated that these effects occurred at the lowest blood Pb levels, compared to other health effects. The evidence reviewed in the current assessment supports and builds upon this conclusion. Small shifts in the population mean IQ can be highly significant from a public health perspective because such shifts could translate into a larger proportion of the population functioning at the low end of the IQ distribution (Section 1.9.1), as well as a smaller proportion of population functioning at the high end of the distribution[1]. Additionally, small Pb-associated increases in the population mean blood pressure could result in an increase in the proportion of the population with hypertension that is significant from a public health perspective.

Air Lead(Pb)-to-Blood Lead(Pb) Relationships

A limited number of epidemiologic studies evaluated relationships between air Pb and blood Pb (Section 1.9.2). Regression models are typically used to produce slopes that estimate the change in blood Pb per change in air Pb concentration (µg/dL per µg Pb/m^3). The larger the slope, the larger is the estimated contribution of air Pb to the blood Pb level in exposed populations.

The range of air-to-blood slope estimates is 4 to 9 µg/dL per µg/m^3 in studies of children. The differences in the estimates across studies, at least in part, reflect the choice of model

[1] This statement follows from the conceptual model described by Weiss et al. (1988), which assumes that the incremental concentration-response between Pb exposure and IQ is similar across the full range of IQ and is not based on actual data.

(e.g., some models predict an increase in the blood Pb-air Pb slope with decreasing air Pb concentration while other models predict a constant blood Pb-air Pb slope across all air Pb concentrations). In addition, differences in the estimates across studies may reflect the different terms that are included in the model (e.g., soil Pb); these terms may account for some of the variation in blood Pb that is attributable to air Pb. Other factors that may explain the variation in the derived blood Pb-air Pb slope include differences in the populations examined and Pb sources (e.g., leaded gasoline or smelter).

Concentration-Response Relationships for Health Effects

Previous assessments found that progressively lower blood Pb levels were associated with cognitive deficits in children, and newly available evidence is generally consistent with findings of the previous review (Section 1.9.3). Compelling evidence for a larger incremental effect of Pb on children's IQ at lower blood Pb levels compared to higher blood Pb levels was presented in the 2006 Pb AQCD based on the international pooled analysis of seven prospective cohort studies, as well as several individual studies. This was supported by a subsequent reanalysis of the pooled data focusing on the shape of the concentration-response function. Several recent studies also support the findings of the original pooled analysis. The majority of the epidemiologic evidence from stratified analyses comparing the lower and the higher ends of the blood Pb distributions also indicates larger effect of Pb on IQ at lower blood Pb levels. The shape of concentration-response relationships is not well characterized for association of health effects with blood or bone Pb concentrations in adults (Section 1.9.3).

Pb Exposure and Neurodevelopmental Deficits in Children

Information about the patterns of exposure that contribute to the blood Pb levels and effects observed in epidemiologic studies is generally lacking. Although, blood Pb may reflect both recent exposures as well as past exposures because Pb is both taken up by and released from the bone, uncertainty regarding the role of recent exposure is greater in adults and older children than in young children who do not have lengthy exposure histories. Several lines of evidence inform the interpretation of epidemiologic studies of young children with regard to the exposures that contribute to observed health effects (Section 1.9.4). Epidemiologic studies find associations of cognitive function with several different blood Pb metrics that represent blood Pb during lifestages or time periods from the prenatal period through adolescence. This epidemiologic evidence is supported by studies of rodents and monkeys indicating that Pb exposures during multiple lifestages and time periods, including prenatal only, prenatal plus lactational, postnatal only, and lifetime are observed to induce impairments in learning. These

findings are consistent with the fact that the nervous system continues to develop throughout childhood.

Potentially At-Risk Populations

The NAAQS are intended to protect public health with an adequate margin of safety. In so doing, protection is provided for both the population as a whole and those groups at increased risk for health effects in response to the air pollutant for which each NAAQS is set. Children are at increased risk for the effects of Pb exposure. Among children, the youngest age groups were observed to be most at risk of elevated blood Pb levels, with levels decreasing with increasing age of the children. Evidence related to childhood and other at-risk factors is described in Section 1.9.6.

Pb Concentrations Corresponding to Ecological Effects

There is limited evidence to relate ambient air concentrations of Pb to levels of deposition onto terrestrial and aquatic ecosystems and to subsequent movement of atmospherically-deposited Pb through environmental compartments (e.g., soil, sediment, water, and biota) (Section 1.9.7). The contribution of atmospheric Pb to specific sites is not clear, and the connection between air concentration of Pb and ecosystem exposure continues to be poorly characterized. Furthermore, the level at which Pb elicits a specific effect is difficult to establish in terrestrial and aquatic systems, due to the influence of other environmental variables (e.g., pH, organic matter) on both Pb bioavailability and toxicity, and also to substantial species differences in Pb sensitivity. Current evidence indicates that Pb is bioaccumulated in biota; however, the sources of Pb in biota have only been identified in a few studies, and the relative contribution of Pb from all sources is usually not known.

Summary

Overall, the evidence evaluated for the current review expands upon findings of the 2006 Pb AQCD and previous assessments, which concluded that there was a strong body of evidence substantiating the health effects from Pb exposure as well as strong evidence of the effects from Pb exposure on some ecological endpoints.

Pb exposure exerts harmful effects on a broad array of organ systems. Cognitive function decrements in children are the effects that are best substantiated as occurring at the lowest blood Pb concentrations (Section 1.6.1.1). There is also a strong body of evidence demonstrating that Pb exposure can cause cardiovascular effects; this evidence strongly suggests that long-term Pb exposure plays a role. Since Pb exposures were generally

higher in the past than they are today, uncertainties exist regarding the relative importance of recent versus past exposure in the development of the Pb-related health effects in the adult populations studied.

With regard to the ecological effects of Pb, uptake of Pb into fauna and subsequent effects on reproduction, growth and survival are established and are further supported by more recent evidence. These may lead to effects at the population, community, and ecosystem level of biological organization. In both terrestrial and aquatic organisms, gradients in response are observed with increasing concentration of Pb and some studies report effects within the range of Pb detected in environmental media over the past several decades. Specifically, effects on reproduction, growth, and survival in sensitive freshwater invertebrates are well-characterized from controlled studies at concentrations at or near Pb concentrations occasionally encountered in U.S. fresh surface waters. Hematological and stress related responses in some terrestrial and aquatic species were also associated with elevated Pb levels in polluted areas. However, in natural environments, modifying factors affect Pb bioavailability and toxicity and there are considerable uncertainties associated with generalizing effects observed in controlled studies to effects at higher levels of biological organization. Furthermore, available studies on community and ecosystem-level effects are usually from contaminated areas where Pb concentrations are much higher than typically encountered in the environment. The contribution of atmospheric Pb to specific sites is not clear and the connection between air concentration of Pb and ecosystem exposure continues to be poorly characterized. Furthermore, the level at which Pb elicits a specific effect is difficult to establish in terrestrial and aquatic systems, due to the influence of other environmental variables (e.g., pH, organic matter) on both Pb bioavailability and toxicity, and also to substantial species differences in Pb sensitivity.

References for Executive Summary

U.S. EPA (U.S. Environmental Protection Agency). (2006b). Air quality criteria for lead: Volume I of II [EPA Report]. (EPA/600/R-05/144aF). Research Triangle Park, NC. http://cfpub.epa.gov/ncea/CFM/recordisplay.cfm?deid=158823

CHAPTER 1 INTEGRATIVE SUMMARY

1.1 ISA Development and Scope

This chapter summarizes and synthesizes the recently available scientific evidence and is intended to provide a concise synopsis of the ISA conclusions and findings that best inform the review of the current NAAQS for lead (Pb). *The Integrated Review Plan (IRP) for the National Ambient Air Quality Standards for Lead* (U.S. EPA, 2011c) identifies a series of policy-relevant questions (in Chapter 3 of the plan) that provide the framework for this assessment. These questions also frame the entire review of the NAAQS for Pb, and thus are informed by both science and policy considerations.

The ISA organizes, presents, and integrates the scientific evidence, which is considered, along with findings from risk analyses and policy considerations, to help the U.S. Environmental Protection Agency (EPA) address these questions during the NAAQS review for Pb. The ISA includes:

- An integration of the evidence on the human health effects associated with Pb exposure, a discussion of important uncertainties identified in the interpretation of the scientific evidence, and an integration across different scientific disciplines and across individual endpoints within major outcome categories.
- An integration of the evidence on the welfare effects[1] of Pb in terrestrial, freshwater and saltwater ecosystems, discussion of endpoints common to plants, invertebrates and vertebrates and consideration of uncertainties in relating atmospheric Pb concentrations to welfare effects.
- An integration of the effects associated with exposure to Pb across the scientific disciplines for health and ecology, focusing on common modes of action.
- Discussion of policy relevant considerations, such as potentially at-risk populations and concentration-response relationships.

EPA has a systematic process for evaluating the scientific evidence and for drawing conclusions and judgments regarding the causal association of air pollution with health and environmental effects. The ISA process includes literature search strategies, criteria for selecting and evaluating studies, approaches for evaluating weight of the evidence, and a framework for making causality determinations. As part of this process, the ISA is reviewed by the public and peer reviewed by a formal panel of scientific experts (the Clean Air Scientific Advisory Committee [CASAC]). The process and causality

[1] Welfare effects as defined in Clean Air Act (CAA) Section 302(h) [42 U.S.C. 7602(h)] include, but are not limited to, "effects on soils, water, crops, vegetation, man-made materials, animals, wildlife, weather, visibility and climate, damage to and deterioration of property, and hazards to transportation, as well as effects on economic values and on personal comfort and well-being."

framework are described in more detail in the Preamble to the ISA. This section provides a brief overview of the process for development of this ISA.

EPA initiated the current review of the Pb NAAQS in February 2010 with a call for information from the public (75 FR 8934). In addition, literature searches were conducted routinely to identify studies published since the last review, focusing on studies published from 2006 (close of the previous scientific assessment) through September 2011. References that were considered for inclusion or actually cited in this ISA can be found at http://hero.epa.gov/lead.

This ISA evaluates relevant epidemiologic, animal toxicological, and welfare effects studies, including those related to concentration-response relationships, mode(s) of action (MOA), and susceptible populations. Additionally, air quality and emissions data, studies on environmental fate and transport, and issues related to Pb toxicokinetics and exposure were considered for inclusion in the document. Previous AQCDs (U.S. EPA, 2006b, 1986b, 1977) have included an extensive body of evidence on these topics. In this ISA, the conclusions and key findings from previous reviews are summarized at the beginning of each section, to provide the foundation for consideration of evidence from recent studies. Results of key studies from previous reviews are included in discussions or tables and figures, as appropriate, and conclusions are drawn based on the synthesis of evidence from recent studies with the extensive literature summarized in previous reviews.

The Preamble discusses the general framework for conducting the science assessment and developing an ISA, including criteria for selecting studies for inclusion in the ISA evaluating and integrating the scientific evidence and developing scientific conclusions. In selecting the studies for inclusion in the Pb ISA, particular emphasis is placed on those studies most relevant to the review of the NAAQS.

In drawing judgments regarding causality for the criteria air pollutants, evidence of health effects in the range of relevant pollutant exposures or doses is considered. Evidence from experimental animal studies observing effects at biomarker levels comparable to, or somewhat above, those currently experienced by the U.S. general population were emphasized. Generally studies with dietary exposures resulting in blood Pb levels within one order of magnitude above the upper end of the distribution of U.S. blood Pb levels were considered in forming conclusions[1] with the majority of studies reporting blood Pb levels below 30 µg/dL. Studies with higher blood Pb levels were considered if they informed the evaluation of modes of action, mechanisms, or kinetics. For toxicological studies where blood Pb levels were not measured, judgments regarding how to

[1] For example, the 95th percentile of the NHANES (2009-2010) distribution of blood Pb level in children 1-5 years old is 3.4 µg/m^3 (CDC, 2013); however, the proportion of individuals with blood Pb levels that exceed this concentration varies depending on factors including age and sex (Section 3.4).

distinguish high from the more relevant low doses were made considering the range of doses across the available body of evidence and emphasizing studies at the lower end of the range.

With respect to the epidemiologic evidence, population-based studies using Pb biomarkers (i.e. blood or bone Pb concentrations) were emphasized with the recognition that many of the U.S populations studied included individuals with higher past than recent Pb exposures. For example, in U.S. population studies during years past (1968-1980) when air concentrations in the U.S. were much higher than they are today, the population geometric mean blood Pb levels were roughly an order of magnitude above current population geometric mean blood Pb levels (Sections 3.4, 5.1, and 4.4.1). Recent occupational studies of populations with relatively high mean blood Pb levels were considered insofar as they addressed a topic area that was of particular relevance to the NAAQS review (e.g., longitudinal studies designed to examine recent versus historical Pb exposure).

Relevant concentrations for drawing causality judgments for the welfare effects of Pb were determined considering the range of Pb concentrations in the environment and the available evidence for concentrations at which effects were observed in plants, invertebrates, and vertebrates. Effects observed at or near ambient Pb concentrations measured in soil, sediment and water in the most recent available studies (Table 1-1) were emphasized and studies generally within one to two orders of magnitude above the reported range of these values were considered in the body of evidence for terrestrial, freshwater and saltwater ecosystems. Studies at higher concentrations were used to the extent that they informed modes of action and illustrated the wide range of sensitivity to Pb across taxa.

The causal determinations for terrestrial, freshwater, and saltwater effects are divided into two categories. The first category includes endpoints that are commonly used in ecological risk assessment (reproduction, growth, and survival). Impacts on these endpoints have the potential to lead to population-level (e.g., abundance, production, extirpation), community-level (e.g., taxa richness, relative abundance) and ecosystem-level effects (Ankley et al., 2010; Suter et al., 2005). The second category includes organism- and sub-organism-level responses such as physiological stress, hematological effects, and neurobehavioral effects. As recognized in EPA's Framework for Ecological Risk Assessment (U.S. EPA, 1992), and in the adverse outcome pathway (AOP) framework (Ankley et al., 2010) endpoints that are measured at one level of biological organization may be related to an endpoint at a higher level. The AOP conceptual framework was proposed to link mechanistic data from initiating events at the molecular level through a series of higher order biological responses to growth, survival and

reproductive endpoints that can be used in ecological risk assessment, i.e., at the population level and higher. In the case of Pb, sub-organismal responses (i.e., physiological stress, hematological effects) and organism-level responses (neurobehavioral alterations) may decrease the overall fitness of an organism, even though their connection to effects at higher levels of biological organization may not have been characterized. Furthermore, the effects of Pb on ecosystems necessarily begin with some initial effects at the molecular level of specific organisms within the ecosystem (U.S. EPA, 1986b). There are many different molecular and cellular level effects, and toxicity of Pb in ecosystems may be attained through multiple modes of action.

The ISA considers evidence of health effects for both short- and long-term pollutant exposures. Since biomarkers are typically used as an index of exposure or dose in epidemiologic studies, there is uncertainty regarding the timing, frequency, level, and duration of the exposure(s) associated with the observed effects and blood Pb (or other biomarker) levels measured in these studies. Some animal toxicological studies provide evidence to inform the exposure patterns that can induce effects in animals and these studies are drawn upon to interpret the human health effects evidence. Exposure regimens used in toxicological studies typically include chronic exposure (i.e., over 10% of the lifespan of the animal), long-term exposure (e.g., greater than 4 weeks in rodents) and acute or short-term exposure (e.g., less than 4 weeks in rodents). For the purpose of this assessment, short-term human exposures are generally defined to include exposures of months (e.g., <one year) while long-term human exposures include those greater than one year in duration. Information including the age of the population studied, study period and study location is also used to aid in the interpretation of findings from epidemiologic studies because Pb exposures have declined over time and exposures vary depending on proximity to Pb sources.

As described in the Preamble (Table II), this ISA uses a five-level hierarchy that classifies the weight of evidence for causation:

- Causal relationship
- Likely to be a causal relationship
- Suggestive of a causal relationship
- Inadequate to infer a causal relationship
- Not likely to be a causal relationship

Briefly, evidence is judged sufficient to conclude that there is a *causal* relationship with relevant Pb exposures when chance, bias and confounding can be ruled out with reasonable confidence. The weight of evidence may be judged “likely to be a causal relationship” or “suggestive of a causal relationship” when important uncertainties

remain. A conclusion of "*likely causal*" may be appropriate if evidence is available from multiple studies or several lines of evidence including cases where the weight of the health effects evidence is largely derived from multiple animal toxicological studies. A conclusion that the evidence is *suggestive* of a causal relationship reflects generally limited evidence, but may include "at least one high-quality epidemiology study" or "a well conducted toxicological study". Evidence is *inadequate* to determine whether a causal relationship exists when the available studies are of insufficient quantity, quality, consistency, or statistical power. If several adequate studies, covering the full range of exposure levels that human beings are known to encounter, considering at-risk populations, are mutually consistent in not showing an effect, the relationship may be judged *not likely to be causal*.

Beyond judgments regarding causality are questions relevant to quantifying health or environmental risks based on the understanding of the quantitative relationships between pollutant exposures and health or welfare effects. Once a determination is made regarding the causal relationship between the pollutant and outcome category, important questions regarding quantitative relationships include:

- What is the concentration-response, exposure-response, or dose-response relationship in the human population?
- What exposure conditions (dose or exposure, exposure pathways, duration and pattern) are important?
- What populations and lifestages appear to be differentially affected i.e., at greater risk of Pb-related health effects?
- What elements of the ecosystem (e.g., types, regions, taxonomic groups, populations, functions, etc.) appear to be affected or are more sensitive to effects?

This ISA is composed of a Preamble, a Legislative and Historical Background, an Executive Summary, and six chapters. Chapter 1 presents an Integrative Summary. Chapter 2 highlights key concepts or issues relevant to understanding the sources, ambient concentrations, and fate and transport of Pb in the environment. Chapter 3 summarizes key concepts and recent findings on Pb exposures, toxicokinetics, and biomarkers reflecting Pb exposure and body burden. Chapter 4 presents a discussion of the MOA of Pb and evaluates and integrates epidemiologic and animal toxicological information on health effects related to Pb exposure. Chapter 5 summarizes the evidence on potentially at-risk populations. Chapter 6 evaluates welfare effects evidence that is relevant to the review of the secondary NAAQS for Pb.

This chapter summarizes and integrates the newly available scientific evidence that best informs consideration of the policy-relevant questions that frame this assessment. The organization of this chapter generally follows the organization of the document as a

whole, with several additional sections including: a discussion of the assessment development and scope (Section 1.1); an integration of the evidence across the disciplines of health and ecology (Section 1.8); a discussion of policy-relevant considerations (Section 1.9); and, an overall summary (Section 1.10).

1.2 Ambient Pb: Source to Concentration

1.2.1 Sources, Fate and Transport of Ambient Pb

The findings of this review build upon those from the 2006 Pb AQCD (U.S. EPA, 2006b), which documented the decline in ambient air Pb emissions following the phase out of alkyl-Pb additives for on-road gasoline and reductions in industrial facility emissions of Pb. Pb emissions declined by 98% from 1970 to 1995 and then by an additional 77% from 1995 to 2008. The 2008 National Emissions Inventory (NEI) reported ambient air Pb emissions of 950 tons. Air Pb emissions represent just a small fraction (by weight) of the Pb processed in U.S. Pb-related industries.

As at the time of the last review, the 2008 NEI (U.S. EPA, 2011a), indicates that piston-engine aircraft emissions comprise the largest share (58%) of total atmospheric Pb emissions in the U.S. Other sources of ambient air Pb, beginning with the next largest, include metal working and mining, fossil fuel combustion, other industrial sources, and miscellaneous sources. On a site-specific basis, emissions are greatest at metal industry sites. Over the period 1991-2010, the amount of Pb used in secondary Pb processing increased by 37%. Exports of Pb increased by 103%, with 2010 exports sent to Mexico as refined Pb; to Canada, China, and Japan in spent Pb-acid batteries; and, to the Republic of Korea as Pb in concentrate (USGS, 2012).

Global atmospheric Pb deposition peaked in the 1970s, followed by a decline (Section 2.2). Pb deposition is greater near Pb emission sources. Both wet and dry deposition are important mechanisms for removing Pb from the atmosphere, and the atmosphere is the main environmental transport media for Pb which is deposited onto surface water and soil. Wet deposition is more important for the fine fraction while the coarse fraction is usually removed by dry deposition. Pb associated with coarse PM deposits to a great extent near local industrial sources, contributing to soil Pb concentrations in those locations, while fine Pb-bearing PM can be transported long distances, contributing Pb contamination in remote areas. Depending on local conditions, once they are deposited, particles may be resuspended and redeposited before reaching a site where further transport is unlikely, especially for dry deposition (Section 2.3). Surface waters act as an important reservoir, with Pb lifetimes in the water column

largely controlled by deposition and resuspension of Pb in sediments. Substantial amounts of Pb may be input to surface waters and sediments by wastewater discharges and through transport of Pb from vehicle wear and building materials in runoff waters without having become airborne. Pb containing sediment particles can be remobilized into the water column (Section 2.3).

1.2.2 Monitoring and Concentrations of Ambient Air Pb

The indicator for the Pb NAAQS is Pb in total suspended particles (Pb-TSP). The Federal Reference Method (FRM) for Pb-TSP specifies that ambient air is drawn through a high-volume TSP sampler onto a glass fiber filter. The Pb-TSP sampler's size selective performance is known to be affected by wind speed and direction, and collection efficiency has been demonstrated to decline with increasing particle size with an uncertain upper size limit (Wedding et al., 1977). There have been only a few studies since the publication of the 2006 Pb AQCD with regard to sampling error in the Pb-TSP FRM or alternatives to the existing Pb-TSP sampling technology. In addition to monitors used historically for sampling Pb-PM, several single stage and multi-stage impactors and inlets used for sampling PM concentrations are also potential options for Pb-PM monitoring when the majority of particles are smaller than 15 μm. Ambient air Pb monitoring requirements have undergone several changes since publication of the 2006 Pb AQCD. The current Pb monitoring network design requirements include two types of FRM monitoring sites: source-oriented and non-source-oriented (Section 2.4). For the purpose of analyzing data for the ISA, monitors reporting to the U.S. EPA Air Quality System (AQS) database were considered to be source-oriented if they were designated in AQS as source-oriented, or if they were located within 1 mile of a 0.5 ton per year or greater source, identified using emissions estimates in the 2005 or 2008 NEI (U.S. EPA, 2008a) (U.S. EPA, 2011a). Source-oriented FRM Pb-TSP monitoring sites are required near sources of air Pb emissions which are expected to or have been shown to contribute to ambient air Pb concentrations in excess of the NAAQS.
Non-source-oriented FRM (Pb-TSP or Pb-PM_{10}) monitoring is also required at national core multipollutant monitoring network (NCore) sites in Core Based Statistical Areas (CBSA) with a population of at least 500,000. In addition to FRM monitoring, Pb is also routinely measured in smaller particle fractions in the chemical speciation network (CSN), interagency monitoring of protected visual environment (IMPROVE), and the national air toxics trends station (NATTS) networks. While monitoring in multiple networks provides extensive geographic coverage, measurements between networks are not directly comparable in all cases because there are differences in the methods,

including the different particle size ranges sampled in the different networks. Depending on monitoring network, Pb is monitored in TSP, PM_{10}, or $PM_{2.5}$.

Ambient air Pb concentrations have declined drastically over the period 1980-2010 (Section 2.5). The median value (across monitoring sties) for the maximum 3-month average concentration within a year has dropped by 97% from 0.87 $\mu g/m^3$ in 1980 to 0.03 $\mu g/m^3$ in 2010. The mean of maximum 3-month average Pb concentrations at source-oriented sites was skewed toward the 75th percentile of the data distribution and exceeded the level of the NAAQS, suggesting that ambient air Pb concentrations are high near a subset of industrial sources of airborne Pb. Studies in the peer-reviewed literature have shown slightly elevated Pb concentrations downwind of industrial sources and airports. Estimates for the natural background Pb concentrations from sources including volcanoes, sea-salt spray, and biogenic sources are ~0.00002 to 0.001 $\mu g/m^3$.

The size distribution of Pb-bearing PM has changed over time and varies by site (Section 2.5.3). Recent study results indicate that the size distribution has generally shifted upward since the 1980s, with the mode of the size distribution of Pb-PM particles now falling between 2.5 μm and 10 μm (Cho et al., 2011). The Pb-PM size distribution depends on whether there are contributions from industrial sources or near-road environments. In contrast to Cho et al. (2011), analysis of the distributional properties of the Pb-PM measured by the AQS monitors, which are often sited near sources, suggests that the largest proportion of particles is still below 2.5 μm in diameter.

1.2.3 Ambient Pb Concentrations in Non-Air Media and Biota

Releases of Pb to the atmosphere have contributed to measurable increases in Pb in rain, snowpack, soil, surface waters, sediments, agricultural plants, livestock, and wildlife across the world, with highest concentrations near Pb sources, such as smelters. After the phase-out of Pb from on-road gasoline and with reductions in industrial emissions, Pb concentrations have decreased considerably in rain, snowpack, and surface waters.

Declining Pb concentrations in tree foliage, trunk sections, and grasses, as well as surface sediments and soils, have also been observed (U.S. EPA, 2006b).

Often, Pb is retained in soils and sediments, where it provides a historical record of deposition. In remote lakes, sediment profiles indicate higher Pb concentrations in near surface sediment as compared to pre-industrial era sediment from greater depth and indicate peak concentrations between 1960 and 1980 (when leaded on-road gasoline was at peak use). Concentrations of Pb in moss, lichens, peat, and aquatic bivalves have been used to understand spatial and temporal distribution patterns of air Pb concentrations.

Ingestion and water intake are the major routes of Pb exposure for aquatic organisms, and food, drinking water, and inhalation are major routes of exposure for livestock and terrestrial wildlife.

Overall, Pb concentrations have decreased substantially in media through which Pb is rapidly transported, such as air and water. Substantial Pb remains in soil and sediment sinks. In areas less affected by major local sources, the highest concentrations are below the surface layers and reflect the phase-out of Pb from on-road gasoline and emission reductions from other sources.

Information on ambient Pb concentrations in non-air media and biota is reported in Section 2.6, and concentrations considered in the interpretation of the ecological evidence are tabulated in Table 1-1. As noted in the Preamble, the ecological causal determinations focus on studies where effects of Pb exposure are observed at or near ambient levels of Pb and studies generally within the range of one to two orders of magnitude above current or ambient conditions were also considered in the body of evidence.

Table 1-1 Pb concentrations in non-air media and biota considered for ecological assessment.

Media	Pb Concentration	Years Data Obtained	References
Soil (non-urban)	Contiguous U.S. Median: 15 mg Pb/kg (dry weight) Contiguous U.S. 95th Percentile: 50 mg Pb/kg (dry weight)	1961-1976	Shaklette (1984)
	National Average: 18.9 mg Pb/kg (dry weight) Range of state averages: 5-38.6 mg Pb/kg (dry weight)	1961-1997	U.S. EPA (2007d, 2006b, 2003b)
Freshwater Sediment	Median: 73 mg Pb/kg (dry weight)	1996-2001	Mahler et al. (2006)
	Median: 28 mg Pb/kg[b] (dry weight)	1991-2003	U.S. EPA (2006b)
Saltwater Sediment	Range: 0.6 to 1,050 mg Pb/kg[a]	Dates not available	Sadiq (1992)
Fresh Surface Water (Dissolved Pb)[b]	Median: 0.50 µg Pb/L[b]; Max: 30 µg Pb/L, 95th percentile 1.1 µg Pb/L	1991-2003	U.S. EPA (2006b)
	Range: 0.0003-0.075 µg Pb/L (Set of National Parks in western U.S.)	2002-2007	Field and Sherrell (2003), U.S. National Park Service (2011)
Saltwater[c]	Range: 0.01–27 µg Pb/L	Dates not available	Sadiq (1992)
Vegetation	Lichens: 0.3-5 mg Pb/kg (dry weight) (Set of National Parks in western U.S.)	2002-2007	U.S. National Park Service (2011)
	Grasses: Geometric Mean: 0.31 kg Pb/kg (dry weight)	1980s-2000s	Vandenhove et al. (2009)
Vertebrates	Fish: Geometric Mean: 0.59 mg Pb/kg (dry weight) (whole fish) Geometric Mean: 0.15 mg Pb/kg (dry weight) (liver) Range: 0.08-22.6 mg Pb/kg (dry weight) (whole fish) Range: 0.01-12.7 mg Pb/kg (dry weight) (liver)	1991-2003	U.S. EPA (2006b)
	Fish (from a set of national parks in western U.S.): 0.0033 (fillet) to 0.97 (liver) mg Pb/kg (dry weight) Moose[d,e]: 0.008-0.029 mg Pb/kg (dry weight) (meat) Moose[d,e]: 0.012-0.023 mg Pb/kg (dry weight) (liver)	2002-2007	U.S. National Park Service (2011)

[a]No information available regarding wet or dry weight

[b]Based on synthesis of National Water-Quality Assessment (NAWQA) data reported in 2006 Pb AQCD (U.S. EPA, 2006b)

[c]Data from a combination of brackish and marine saltwater samples. In general, Pb in seawater is higher in coastal areas and estuaries since these locations are closer to sources of Pb contamination and loading from terrestrial systems.

[d] The reference cited and its source citations show that observations date from studies published in 1977-1990, indicating that the data were obtained no later than those years. Further, these measurements seem to be for non-U.S. locations, including the max, which is well above other reported values in these refs.

[e]Three moose in one Alaskan park

1.3 Exposure to Ambient Pb

Human Pb exposure is difficult to assess because Pb has multiple sources in the environment and passes through various media (Section 3.1). Air-related pathways of Pb exposure are the focus of this assessment. In addition to inhalation of Pb in ambient air, air-related Pb exposure pathways include inhalation and ingestion of Pb in indoor dust and/or outdoor soil that originated from recent or historic ambient air (e.g., air Pb that has penetrated into the residence either via the air or tracking of soil), ingestion of Pb in drinking water drawn from surface water contaminated from atmospheric deposition or contaminated from surface runoff of deposited Pb, and ingestion of Pb in dietary sources after uptake by plants or grazing animals. Soil can act as a reservoir for deposited Pb emissions. Exposure to soil contaminated with deposited Pb can occur through resuspended PM as well as hand-to-mouth contact, which is the main pathway of childhood air-related exposure to Pb. The primary contribution of ambient air Pb to young children's blood Pb concentrations is generally due to ingestion of Pb following its deposition in soils and dusts rather than inhalation of ambient air (Section 3.1.1.2). Non-ambient air-related exposures include hand-to-mouth contact with dust or chips of peeling Pb-containing paint, or ingestion of Pb in drinking water conveyed through Pb pipes. Several study results indicate that Pb-containing paint in the home and home age (often a surrogate for the presence of Pb paint) are important residential factors that increase risk of elevated blood Pb (Sections 1.9.6 and 5.2.6). Most Pb biomarker studies do not indicate species or isotopic signature. As a consequence, non-air exposures are reviewed in this section, because they can also contribute to Pb body burden.

A number of monitoring and modeling techniques have been employed for ambient Pb exposure assessment. Environmental Pb concentration data can be collected from ambient air Pb monitors, soil Pb samples, dust Pb samples, and dietary Pb samples to estimate human exposure. Exposure estimation error depends in part on the collection efficiency of these methods; collection efficiency for ambient air Pb FRM samplers is described in Section 2.4. Models, such as the Integrated Exposure Uptake Biokinetic (IEUBK) model, simulate human exposure to Pb from multiple sources and through various routes including inhalation and ingestion. IEUBK model inputs include soil-Pb concentration, air-Pb concentration, dietary-Pb intake including drinking water, Pb-dust ingestion, human activity, and biokinetic factors. The relative contribution from specific exposure pathways (e.g., water, diet, soil, ambient air) to blood Pb concentrations is situation specific. Measurements and/or assumptions can be utilized when formulating the model inputs; errors in measurements and assumptions thus have the potential to propagate through exposure models.

The size distribution of dust particles containing Pb differs from the size distribution of inhalable ambient Pb-bearing PM (Sections 2.5 and 3.1). Airborne particles containing Pb tend to be small (much of the distribution <10 µm) compared with soil or dust particles containing Pb (~50 µm to several hundred µm). Ingestion through hand-to-mouth contact is the predominant exposure pathway for the larger particles in soil and dust containing Pb.

1.4 Toxicokinetics

The majority of Pb in the body is found in bone (roughly 90% in adults, 70% in children); only about 1% of Pb is found in the blood. Pb in blood is primarily (~99%) bound to red blood cells (RBCs). It has been suggested that the small fraction of Pb in plasma (<1%) may be the more biologically labile and toxicologically active fraction of the circulating Pb. The relationship between Pb in blood and plasma is pseudo-linear at relatively low daily Pb intakes (i.e., <10 µg/kg per day) and at blood Pb concentrations <25 µg/dL, and becomes curvilinear at higher blood Pb concentrations due to saturable binding to RBC proteins. As blood Pb level increases and the higher affinity binding sites for Pb in RBCs become saturated, a larger fraction of the blood Pb is available in plasma to distribute to brain and other Pb-responsive tissues. See Section 3.2 for additional details.

The burden of Pb in the body may be viewed as divided between a dominant slow (i.e., uptake and elimination) compartment (bone) and smaller fast compartment(s) (soft tissues). Pb uptake to and elimination from soft tissues is much faster than in bone. Pb accumulates in bone regions undergoing the most active calcification at the time of exposure. During infancy and childhood, bone calcification is most active in trabecular bone (e.g., patella); whereas, in adulthood, calcification occurs at sites of remodeling in cortical (e.g., tibia) and trabecular bone (Aufderheide and Wittmers, 1992). A high bone formation rate in early childhood results in the rapid uptake of circulating Pb into mineralizing bone; however, in early childhood bone Pb is also recycled to other tissue compartments or excreted in accordance with a high bone resorption rate (O'Flaherty, 1995). Thus, much of the Pb acquired early in life is not permanently fixed in the bone.

The exchange of Pb from plasma to the bone surface is a relatively rapid process. Pb in bone becomes distributed in trabecular and the more dense cortical bone. The proportion of cortical to trabecular bone in the human body varies by age, but on average is about 80% cortical to 20% trabecular. Of the bone types, trabecular bone is more reflective of recent exposures than is cortical bone due to the slow turnover rate and lower blood perfusion of cortical bone. Some Pb diffuses to kinetically deeper bone regions where it is relatively inert, particularly in adults. These bone compartments are much more labile

in infants and children than in adults as reflected by half-times for movement of Pb from bone into the plasma (e.g., cortical half-time = 0.23 years at birth, 3.7 years at 15 years of age, and 23 years in adults; trabecular half-time = 0.23 years at birth, 2.0 years at 15 years of age, and 3.8 years in adults) (Leggett, 1993). See Section 3.2 for additional details.

Evidence for maternal-to-fetal transfer of Pb in humans is derived from cord blood to maternal blood Pb ratios (i.e., cord blood Pb concentration divided by mother's blood Pb). Group mean ratios range from about 0.7 to 1.0 at the time of delivery for mean maternal blood Pb levels ranging from 1.7 to 8.6 µg/dL. Transplacental transfer of Pb may be facilitated by an increase in the plasma/blood Pb concentration ratio during pregnancy. Maternal-to-fetal transfer of Pb appears to be related partly to the mobilization of Pb from the maternal skeleton. See Section 3.2.2.4 for additional details.

The dominant elimination phase of Pb kinetics in the blood, exhibited shortly after a change in exposure occurs, has a half-life of ~20-30 days. An abrupt change in Pb uptake gives rise to a relatively rapid change in blood Pb, to a new quasi-steady state, achieved in ~75-100 days (i.e., 3-4 times the blood elimination half-life). A slower phase of Pb clearance from the blood may become evident with longer observation periods following a decrease in exposure due to the gradual redistribution of Pb among bone and other compartments. See Section 3.3 for additional details.

1.5 Pb Biomarkers

Overall, blood Pb levels have been decreasing among U.S. children and adults over the past 35 years (Section 3.4). The median blood Pb level for the entire U.S. population is 1.1 µg/dL and the 95th percentile blood Pb level is 3.3 µg/dL, based on the 2009-2010 National Health and Nutrition Examination Survey (NHANES) data (CDC, 2013). Among children aged 1-5 years, the median and 95th percentiles were slightly higher, at 1.2 µg/dL and 3.4 µg/dL, respectively.

Blood Pb is dependent on both the recent exposure history of the individual, as well as the long-term exposure history that determines body burden and Pb in bone. The contribution of bone Pb to blood Pb changes, depending on the duration and intensity of the exposure, age, and various other physiological stressors (e.g., nutritional status, pregnancy, menopause, extended bed rest, hyperparathyroidism) that may affect bone remodeling, which normally and continuously occurs. In children, largely due to faster exchange of Pb to and from bone, blood Pb is both an index of recent exposure and potentially an index of body burden. In adults and children whose exposure to Pb has effectively ceased or greatly decreased, there is a rapid decline in blood Pb over the first

few months followed by a more gradual, slow decline in blood Pb concentrations over the period of years due to the gradual release of Pb from bone. Bone Pb is an index of cumulative exposure and body burden. Even bone compartments should be recognized as reflective of differing exposure periods with Pb in trabecular bone exchanging more rapidly than Pb in cortical bone with the blood. Consequently, Pb in cortical bone is a better marker of cumulative exposure, while Pb in trabecular bone is more likely to be correlated with blood Pb, even in adults. See Section 3.3 for additional details.

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

The concentration of Pb in urine follows blood Pb concentration. There is added complexity with Pb in urine because concentration is also dependent upon urine flow rate, which requires timed urine samples that is often not feasible in epidemiologic studies. Other biomarkers have been utilized to a lesser extent (e.g., Pb in teeth). See Section 3.3.

1.6 Health Effects

This section summarizes and evaluates the evidence from toxicological and epidemiologic studies of the health effects associated with Pb exposure and integrates that evidence across these disciplines. The coherence of the findings from experimental animal and epidemiologic studies, including evidence for potential MOA, is evaluated to establish biological plausibility and address uncertainties in the epidemiologic evidence due to biases from factors such as reverse causality and confounding. Both short- and long-term Pb exposures are considered (Section 1.1); information on the frequency, timing, level and duration of exposure in animal toxicological studies is used to inform

the interpretation of epidemiologic studies regarding the relevant patterns of exposure that are likely to be associated with the health effects.

The health evidence is organized into groups of related endpoints (e.g., cognitive function, externalizing behaviors, neurodegenerative diseases). This evidence is considered in combination with the evidence from other fields (e.g., toxicokinetics) and weighed against the attributes described in the framework for causal determination (Table II of the Preamble) to draw conclusions regarding the causal relationship between Pb exposure and the health effects evaluated in this assessment. A more detailed discussion of the underlying evidence used to formulate each causal determination can be found in Chapter 5 of this document. Table 1-2 summarizes the conclusions formed regarding the causal relationships between Pb exposure and health effects.

Table 1-2 Summary of causal determinations for the relationship between exposure to Pb and health effects.

Health Outcome	Causality Determination[a] (Table with Key Evidence)
Nervous System Effects (Section 4.3.15)	
Children – Nervous System Effects	
Cognitive Function Decrements	Causal Relationship (Table 4-17)
Clear evidence of cognitive function decrements (as measured by Full Scale IQ, academic performance, and executive function) in young children (4 to 11 years old) with mean or group blood Pb levels measured at various lifestages and time periods between 2 and 8 µg/dL. Clear support from animal toxicological studies that demonstrate decrements in learning, memory, and executive function with dietary exposures resulting in relevant blood Pb levels of 10-25 µg/dL. Plausible MOAs are demonstrated.	
Externalizing Behaviors: Attention, Impulsivity and Hyperactivity[b,d, e]	Causal Relationship (Table 4-17)
Clear evidence of attention decrements, impulsivity and hyperactivity (assessed using objective neuropsychological tests and parent and teacher ratings) in children 7-17 years and young adults ages 19-20 years. The strongest evidence for blood Pb-associated increases in these behaviors was found in prospective studies examining prenatal (maternal or cord), age 3-60 months, age 6 years, or lifetime average (to age 11-13 years) mean blood Pb levels of 7 to 14 µg/dL and groups with early childhood (age 30 months) blood Pb levels >10 µg/dL. Biological plausibility is provided by animal toxicological studies demonstrating impulsivity or impaired response inhibition with relevant prenatal, lactational, post-lactational and lifetime Pb exposures. Plausible MOAs are demonstrated.	

Table 1-2 (Continued): Summary of causal determinations for the relationship between exposure to Pb and health effects.

Health Outcome	Causality Determination[a] (Table with Key Evidence)
Externalizing Behaviors: Conduct Disorders in Children and Young Adults[c, d]	Likely Causal Relationship (Table 4-17)
Prospective epidemiologic studies find that early childhood (age 30 months, 6 years) or lifetime average (to age 11-13 years) blood Pb levels or tooth Pb levels (from ages 6-8 years) are associated with criminal offenses in young adults ages 19-24 years and with higher parent and teacher ratings of behaviors related to conduct disorders in children ages 8-17 years. Pb-associated increases in conduct disorders were found in populations with mean blood Pb levels 7 to14 µg/dL; associations with lower blood Pb levels as observed in cross-sectional studies were likely to be influenced by higher earlier Pb exposures. There is coherence in epidemiologic findings among related measures of conduct disorders. Evidence of Pb induced aggression in animals was mixed, with increases in aggression found in some studies of adult animals with gestational plus lifetime Pb exposure but not juvenile animals. The lack of clear biological plausibility produces some uncertainty.	
Internalizing Behaviors	Likely Causal Relationship (Table 4-17)
Prospective epidemiologic studies find associations of higher lifetime average blood (mean: ~14 µg/dL) or childhood tooth (from ages 6-8 years) Pb levels with higher parent and teacher ratings of internalizing behaviors such as symptoms of depression or anxiety, and withdrawn behavior in children ages 8-13 years. Consideration of potential confounding by parental caregiving was not consistent and findings from cross-sectional studies in populations ages 5 and 7 years with mean blood Pb levels of 5 µg/dL were mixed. Animal toxicological studies demonstrate depression-like behaviors and increases in emotionality with relevant lactational exposures. Plausible MOAs are demonstrated.	
Auditory Function Decrements	Likely Causal Relationship (Table 4-17)
A prospective epidemiologic study and large cross-sectional studies indicate associations between blood Pb levels and increased hearing thresholds at ages 4-19 years. Across studies, associations were found with blood Pb levels measured at various time periods, including prenatal maternal, neonatal (10 day, mean 4.8 µg/dL), lifetime average, and concurrent (ages 4-19 years) blood Pb levels (median 8 µg/dL). Plausible MOAs are demonstrated. The lack of biological plausibility in animals with relevant exposures produces some uncertainty.	
Visual Function Decrements	Inadequate to Infer a Causal Relationship (Table 4-17)
The available epidemiologic and toxicological evidence is of insufficient, quantity, quality and consistency.	
Motor Function Decrements	Likely Causal Relationship (Table 4-17)
Prospective epidemiologic studies provide evidence of associations of fine and gross motor function decrements in children ages 4-17 years with lifetime average blood Pb levels and with blood Pb levels measured at various time periods with means generally ranging from 4.8 to 12 µg/dL. Results were inconsistent in cross sectional studies with concurrent blood Pb level means 2-5 µg/dL. Limited evidence in animal toxicological studies with relevant Pb exposures.	
Adults – Nervous System Effects	
Cognitive Function Decrements	Likely Causal Relationship (Table 4-17)
Prospective studies indicate associations of higher baseline bone Pb levels with declines in cognitive function (executive function, visuospatial skills, learning and memory) in adults (>age 50 years) over 2- to 4-year periods. Cross-sectional studies provide additional support. Uncertainties remain regarding the timing, frequency, duration and level of the Pb exposures contributing to the effects observed and residual confounding by age. Biological plausibility is provided by findings that relevant lifetime Pb exposures from gestation, birth, or after weaning induce learning impairments in adult animals and by evidence demonstrating plausible MOAs.	
Psychopathological Effects	Likely Causal Relationship (Table 4-17)
Cross-sectional studies in a few populations demonstrate associations of higher concurrent blood or tibia Pb levels with self-reported symptoms of depression and anxiety in adults. Uncertainties remain regarding the timing, frequency, duration and level of Pb exposures contributing to the observed associations and residual confounding by age. Observations of depression-like behavior in animals with dietary lactational Pb exposure, with some evidence at relevant blood Pb levels, and evidence demonstrating plausible MOAs in experimental animals provides support.	

Table 1-2 (Continued): Summary of causal determinations for the relationship between exposure to Pb and health effects.

Health Outcome	Causality Determination[a] (Table with Key Evidence)
Auditory Function Decrements	Suggestive of a Causal Relationship (Table 4-17)
A high-quality prospective epidemiologic study finds associations of higher tibia Pb level with a greater rate of elevations in hearing threshold over 20 years. Some evidence indicates effects on relevant MOAs but important uncertainties remain related to effects on auditory function in animals with relevant Pb exposures.	
Visual Function Decrements	Inadequate to Infer a Causal Relationship (Table 4-17)
The available epidemiologic and toxicological evidence is of insufficient, quantity, quality and consistency.	
Neurodegenerative Diseases	Inadequate to Infer a Causal Relationship (Table 4-17)
The available epidemiologic and toxicological evidence is of insufficient, quantity, quality and consistency.	
Cardiovascular Effects (Section 4.4.7)	
Hypertension	Causal Relationship (Table 4-24)
Prospective epidemiologic studies with adjustment for multiple potential confounders consistently find associations of blood and bone Pb levels with hypertension incidence and increased blood pressure (BP) in adults. Cross-sectional studies provide supporting evidence. Meta-analyses underscore the consistency and reproducibility of the Pb associated increase in blood pressure and hypertension (a doubling of concurrent blood Pb level (between 1 and 40 µg/dL) is associated with a 1 mmHg increase in systolic BP); however, uncertainties remain regarding the timing, frequency, duration and level of Pb exposures contributing to the effects observed in epidemiologic studies. Experimental animal studies demonstrate effects on BP after long-term Pb exposure resulting in mean blood Pb levels of 10 µg/dL or greater. Plausible MOAs are demonstrated.	
Subclinical Atherosclerosis	Suggestive of a Causal Relationship (Table 4-24)
Cross-sectional analyses of NHANES data find associations of blood Pb level with peripheral artery disease (PAD) in adults. Animal toxicological evidence is limited to studies of MOA (oxidative stress, inflammation, endothelial cell dysfunction) that demonstrate biologically plausible mechanisms through which Pb exposure may initiate atherosclerotic vessel disease.	
Coronary Heart Disease	Causal Relationship (Table 4-24)
Prospective epidemiologic studies consistently find associations of Pb biomarkers with cardiovascular mortality and morbidity, specifically myocardial infarction (MI), ischemic heart disease (IHD), or HRV; however, uncertainties remain regarding the timing, frequency, duration and level of Pb exposures contributing to the effects observed in epidemiologic studies. Thrombus formation was observed in animals after relevant long term exposure and MOAs (hypertension, decreased HRV, increased corrected QT (QTc) interval, and corrected QRS complex (QRSc) duration in electrocardiogram [ECG]) are demonstrated in humans and animals.	
Cerebrovascular Disease	Inadequate to Infer a Causal Relationship (Table 4-24)
The available epidemiologic and toxicological evidence is of insufficient, quantity, quality, and/or consistency. Plausible MOAs, which are shared with hypertension and atherosclerosis, are demonstrated.	
Renal Effects (Section 4.5.5)	
Reduced Kidney Function	Suggestive of a Causal Relationship (Table 4-31)
Multiple high quality epidemiologic studies provide evidence that Pb exposure is associated with reduced kidney function; however, uncertainty remains regarding the potential for reverse causality to explain findings in humans. Further, inconsistencies and limitations in occupational studies, epidemiologic studies of children and clinical trials of chelation of CKD patient preclude strong inferences to be drawn based on their results. Although longitudinal studies found Pb-associated decrements in renal function in populations with mean blood Pb levels of 7 and 9 µg/dL, the contributions of higher past Pb exposures cannot be excluded. Animal toxicological studies demonstrate Pb-induced kidney dysfunction at blood Pb levels greater than 30 µg/dL; however, evidence in animals with blood Pb levels < 20 µg/dL is generally not available. At blood Pb levels between 20 and 30 µg/dL studies provide some evidence for dysfunction in kidney function measures (e.g., decreased creatinine clearance, increased serum creatinine, increased BUN). Plausible MOAs (Pb induced hypertension, renal oxidative stress and inflammation, morphological changes, and increased uric acid) are demonstrated.	

Table 1-2 (Continued): Summary of causal determinations for the relationship between exposure to Pb and health effects.

Health Outcome	Causality Determination[a] (Table with Key Evidence)
Immune System Effects (Section 4.6.8)	
Atopic and Inflammatory Responses	Likely Causal Relationship (Table 4-34)
Prospective studies of children ages 1-5 years indicate associations of prenatal cord and childhood blood Pb levels with asthma and allergy. This evidence is supported by cross-sectional associations between higher concurrent blood Pb levels (>10 µg/dL) in children and higher IgE. Uncertainties related to potential confounding by SES, smoking or allergen exposure are reduced through consideration of the evidence from experimental animal studies. The biological plausibility for the effects of Pb on IgE is provided by consistent findings in animals with gestational or gestational-lactational Pb exposures, with some evidence at blood Pb levels relevant to humans. Strong evidence of Pb-induced increases in Th2 cytokine production and inflammation in animals demonstrates MOA.	
Decreased Host Resistance	Likely Causal Relationship (Table 4-34)
Animal toxicological studies provide the majority of the evidence for Pb-induced decreased host resistance. Dietary Pb exposure producing relevant blood Pb levels (7-25 µg/dL) results in increased susceptibility to bacterial infection and suppressed delayed type hypersensitivity. Further, evidence demonstrating plausible MOA, including suppressed production of Th1 cytokines and decreased macrophage function in animals, provides coherence.	
Autoimmunity	Inadequate to Infer a Causal Relationship (Table 4-34)
The available toxicological and epidemiologic studies do not sufficiently inform Pb-induced generation of auto-antibodies with relevant Pb exposures.	
Hematologic Effects (Section 4.7.4)	
Decreased Red Blood Cell (RBC) Survival and Function	Causal Relationship (Table 4-35)
Animal toxicological studies demonstrate that exposures resulting in blood Pb levels relevant to humans (2-7 µg/dL) alter several hematological parameters (Hemoglobin [Hb], Hematocrit [Hct], and mean corpuscular volume [MCV]),increase measures of oxidative stress and increase cytotoxicity in red blood cell (RBC) precursor cells. Limited body of epidemiologic studies provides additional support for the association of Pb exposure with these endpoints. Plausible MOAs are demonstrated in experimental animals.	
Altered Heme Synthesis	Causal Relationship (Table 4-35)
Consistent findings from studies in experimental adult animal studies report that relevant exposures (e.g. blood Pb levels of 6.5 µg/dL) cause decreased ALAD and ferrochelatase activities. Additional support is garnered from a larger body of ecotoxicological studies demonstrating decreased ALAD activity across a wide range of species and a limited body of epidemiologic studies. Plausible MOAs are demonstrated in experimental animals.	
Reproductive and Developmental Effects (Section 4.8.5)	
Development	Causal Relationship (Table 4-48)
Multiple cross-sectional epidemiologic studies report associations between concurrent blood Pb levels and delayed pubertal onset for girls (6-18 years) and boys (8-15 years). These associations are consistently observed in populations with concurrent blood Pb levels 1.2-9.5 µg/dL. Few studies consider confounding by nutrition. Uncertainties remain regarding the timing, frequency, duration and level of Pb exposures contributing to the effects observed in epidemiologic studies of older children.Experimental animal studies demonstrate delayed onset of puberty in female pups with blood Pb levels of 1.3-13 µg/dL and delayed male sexual maturity at blood Pb levels of 34 µg/dL.	
Birth Outcomes (e.g., low birth weight, spontaneous abortion)	Suggestive of Causal Relationship (Table 4-48)
Some well-conducted epidemiologic studies report associations of maternal Pb biomarkers or cord blood Pb with preterm birth and low birth weight/fetal growth; however, the epidemiologic evidence is inconsistent overall and findings from experimental animal studies are mixed.	

Table 1-2 (Continued): Summary of causal determinations for the relationship between exposure to Pb and health effects.

Health Outcome	Causality Determination[a] (Table with Key Evidence)
Male Reproductive Function	Causal Relationship (Table 4-48)
Key evidence is provided by toxicological studies in rodents, non-human primates, and rabbits showing detrimental effects on semen quality, sperm and fecundity/fertility with supporting evidence in epidemiologic studies. Toxicological studies with relevant Pb exposure routes leading to blood Pb concentrations ranging from 5-43 µg/dL reported effects on sperm quality and sperm production rate, sperm DNA damage, and histological or ultrastructural damage to the male reproductive organs. Consistent associations in studies of occupational populations with concurrent blood Pb levels of 25 µg/dL and greater, report detrimental effects of Pb on sperm; however, uncertainties remain regarding the timing, frequency, duration and level of Pb exposures contributing to the effects observed in epidemiologic studies.	
Female Reproductive Function	Suggestive of Causal Relationship (Table 4-48)
Although findings are mixed overall, the body of evidence include some high-quality epidemiologic and toxicological studies, suggesting that Pb may affect some aspects of female reproductive function (hormone level, placental pathology).	
Cancer (Section 4.10.5)	
Cancer	Likely Causal Relationship (Table 4-50)
The animal toxicological literature provides the strong evidence for long-term exposure (i.e., 18 months or 2 years) to high concentrations of Pb (> 2,600 ppm) inducing tumor development; findings from epidemiologic studies inconsistent. Plausible MOAs are demonstrated.	

[a] In drawing conclusions regarding the causal relationship between Pb exposure and human health effects, evidence in the range of relevant pollutant exposures or biomarker levels was considered. Specifically, population-based epidemiology studies were emphasized with the recognition that many of the U.S populations studied included individuals with higher past than recent Pb exposures. Evidence from toxicological studies of effects observed in experimental animals at biomarker levels (e.g. blood Pb) comparable to those currently experienced by the U.S. general population were emphasized. Generally, studies with dietary exposures resulting in blood Pb levels within one order of magnitude above the upper end of the distribution of U.S. blood Pb levels were considered in forming concusions, with the majority of studies reporting blood Pb levels below 30 µg/dL. Studies with higher blood Pb levels were considered if they informed the evaluation of MOA, mechanisms, or kinetics. (Preamble, Section 1.1).

[b] Within the attention deficit hyperactivity disorder domain of externalizing behaviors, studies of Pb exposure have focused primarily on attention, impulsivity, and hyperactivity. Because the studies of ADHD were limited in terms of their design and did not adequately consider potential confounding by factors such as SES, parental education, or parental caregiving quality, they were not a major consideration in drawing conclusions about the relationship between Pb exposure and attention, impulsivity, and hyperactivity.

[c] Two domains of conduct disorders,(i.e., undersocialized aggressive conduct disorder and socialized aggressive conduct disorder), are combined for the purpose of this assessment because it is difficult to differentiate between these two domains in the available epidemiologic studies, which examine multiple endpoints such as delinquent behavior, aggression, antisocial behavior. Criminal offenses are included in the evaluation because they can be predicted by earlier conduct disorders (Section 4.3.3.2).

[d] There was limited evaluation of potential confounding by parental psychopathology, which is a strong risk factor for externalizing behaviors, in the majority of the epidemiologic studies; however, evidence of an association of between psychopathology in parents and Pb exposure in their children is not available (Section 4.3.3).

[e] Strong evidence from experimental animal studies reduces uncertainty related to confounding generally.

1.6.1 Nervous System Effects

The collective body of epidemiologic and toxicological evidence integrated across that reviewed in the 2006 Pb AQCD (U.S. EPA, 2006b) coupled with recently available data demonstrates the effects of Pb exposure on a range of nervous system effects. In children, these effects include cognitive function (Sections 4.3.2.1, 4.3.2.2, 4.3.2.3, 4.3.2.4, 4.3.2.5), externalizing behaviors (Section 4.3.3), internalizing behaviors (Section 4.3.4), auditory function (Section 4.3.6.1), visual function (Section 4.3.6.2), and motor function (Section 4.3.7). In adults, nervous system effects examined in relation to Pb exposure include cognitive function (Section 4.3.2.7), psychopathological effects (Section 4.3.5), auditory function (Section 4.3.6.1), visual function (Section 4.3.6.2), and neurodegenerative diseases (Section 4.3.9).

1.6.1.1 Children

Cognitive Function Decrements

Multiple prospective studies conducted in diverse populations consistently demonstrate associations of higher blood and tooth Pb levels with lower full scale IQ (FSIQ), executive function, and academic performance and achievement. Most studies examined representative populations and had moderate to high follow-up participation without indication of selective participation among children with higher blood Pb levels and lower cognitive function. Associations between blood Pb level and cognitive function decrements were found with adjustment for several potential confounding factors, most commonly, socioeconomic status (SES), parental IQ, parental education, and parental caregiving quality. In children ages 4-11 years, associations were found with prenatal, early childhood, childhood average, and concurrent blood Pb levels in populations with mean or group blood Pb levels in the range 2-8 µg/dL (Section 4.3.2). Neither epidemiologic nor toxicological evidence has identified an individual critical lifestage or duration of Pb exposure within childhood that is associated with cognitive function decrements. Several epidemiologic studies found a supralinear concentration-response relationship. A threshold for cognitive function decrements is not discernable from the available evidence (i.e., examination of early childhood blood Pb or concurrent [with peak <10 µg/dL] blood Pb in the range of < 1 to 10 µg/dL). Evidence in children was clearly supported by observations of Pb-induced impairments in learning, memory, and executive function in juvenile animals. Several studies in animals indicated learning impairments with prenatal, lactational, post-lactational and lifetime (with or without

prenatal) Pb exposures that resulted in blood Pb levels of 10-25 µg/dL. The mode of action for Pb-associated cognitive function decrements is supported by observations of Pb-induced impairments in neurogenesis, synaptogenesis and synaptic pruning, long term potentiation, and neurotransmitter function in the hippocampus, prefrontal cortex, and nucleus accumbens. The associations consistently found for FSIQ and other measures of cognitive function in prospective studies of children with adjustment for SES, parental education, and parental caregiving quality and the biological plausibility provided by evidence in animals for impairments in learning, memory, and executive function with relevant Pb exposures and evidence describing modes of action is sufficient to conclude that there is a causal relationship between Pb exposure and decrements in cognitive function in children.

Externalizing Behaviors

There are three domains of externalizing behaviors (Section 4.3.3). These domains are attention deficit hyperactivity disorder, undersocialized aggressive conduct disorder, and socialized aggressive conduct disorder. Studies of the effect of Pb exposure on the domain of attention deficit hyperactivity disorder have focused primarily on attention, impulsivity, and hyperactivity not diagnosis of ADHD. For the purpose of this assessment, the two domains of conduct disorders are combined because it is difficult to differentiate between these two domains based on the available epidemiologic studies of Pb exposure, which examine multiple endpoints such as delinquent behavior, aggression, and antisocial behavior. Criminal offenses are included within domain because they can be predicted by earlier conduct disorders.

Attention, Impulsivity and Hyperactivity

Although examined less extensively than cognitive function, several prospective studies demonstrated associations of blood or tooth Pb levels measured years before outcomes with attention decrements and hyperactivity in children 7-17 years and young adults ages 19-20 years as assessed using objective neuropsychological tests and rated by parents and teachers. Most of these prospective studies examined representative populations without indication of participation conditional on blood Pb levels and behavior. The results from prospective studies were adjusted for potential confounding by SES as well as parental education and caregiving quality, with some studies also considering parental cognitive function, birth outcomes, substance abuse, and nutritional factors. In prospective studies, blood Pb-associated attention decrements and hyperactivity were found in populations with prenatal (maternal or cord), age 3-60 month average, age 6 year, or lifetime average (to age 11-13 years) mean blood Pb levels of 7 to 14 µg/dL and groups with age 30 month blood Pb levels >10 µg/dL. Most well-conducted cross-sectional studies that

examined several potential confounding factors found associations of attention decrements, impulsivity, and hyperactivity in children ages 5-7.5 years with concurrent blood Pb levels with means of 5-5.4 µg/dL but cannot establish temporality or exclude the influence of higher blood Pb levels earlier in childhood. Biological plausibility for observations in children is provided by several findings in animals for increases in impulsivity or impaired response inhibition with relevant post-weaning and lifetime Pb exposures that resulted in blood Pb levels of 11 to 30 µg/dL. The mode of action for Pb-associated attention decrements, impulsivity, and hyperactivity is supported by observations of Pb-induced impairments in neurogenesis, synaptic pruning, and dopamine transmission in the prefrontal cerebral cortex, cerebellum, and hippocampus. The consistency of epidemiologic evidence for attention decrements and hyperactivity from prospective studies and the biological plausibility provided by evidence for Pb-induced impulsivity in animals and for underlying modes of action is sufficient to conclude that there is a causal relationship between Pb exposure and effects on attention, impulsivity, and hyperactivity in children.

Conduct Disorders in Children and Young Adults

Prospective studies consistently indicate that earlier childhood (e.g., age 30 months, 6 years) or lifetime average (to age 11-13 years) blood Pb levels or tooth (from ages 6-8 years) Pb levels are associated with criminal offenses in young adults ages 19-24 years and with higher parent and teacher ratings of behaviors related to conduct disorders in children ages 7-17 years. Pb-associated increases in conduct disorders were found in populations with mean blood Pb levels 7-14 µg/dL. Associations with lower blood Pb levels that are not influenced by higher earlier Pb exposures are not well characterized. These associations were found without indication of strong selection bias and with adjustment for SES, parental education and IQ, parental caregiving quality, family functioning, smoking, and substance abuse. Supporting evidence is provided by cross-sectional evidence of children participating in NHANES and a meta-analysis of prospective and cross-sectional studies. Additionally, there is coherence in epidemiologic findings among related measures of conduct disorders. Evidence for Pb-induced aggression in animals is mixed with increases in aggression found in some studies of adult animals with gestational plus lifetime Pb exposure but not juvenile animals. The consistent epidemiologic evidence from prospective and cross-sectional studies for criminal offenses and ratings of behaviors related to conduct disorders but uncertainty due to lack of clear evidence for aggression in animals is sufficient to conclude that a causal relationship is likely to exist between Pb exposure and conduct disorders in children and young adults.

Internalizing Behaviors

Prospective studies in a few populations demonstrate associations of higher lifetime average blood (mean: ~14 µg/dL) or childhood tooth (from ages 6-8 years) Pb levels with higher parent and teacher ratings of internalizing behaviors such withdrawn behavior and symptoms of depression and anxiety in children ages 8-13 years. The lack of selective participation by blood Pb level and associations found with parental and teacher ratings do not provide strong indication of biased reporting of behaviors for children with higher blood Pb levels. The few cross-sectional associations in populations with mean concurrent blood Pb levels of 5 µg/dL were inconsistent. Pb-associated increases in internalizing behaviors were found with adjustment for maternal education and SES-related variables. Consideration for potential confounding by parental caregiving quality was inconsistent. Despite some uncertainty in the epidemiologic evidence, the biological plausibility for the effects of Pb on internalizing behaviors is provided by a few findings in animals with dietary lactational Pb exposure, with some evidence at blood Pb levels relevant to humans. Additional toxicological evidence supports modes of action, including Pb-induced changes in the HPA axis and dopaminergic and GABAergic systems. The evidence from prospective studies in a few populations of children and the coherence with evidence from a few animal studies with relevant Pb exposures and mode of action but some uncertainty related to potential confounding by parental caregiving quality in studies of children is sufficient to conclude that a causal relationship is likely to exist between Pb exposure and internalizing behaviors in children.

Auditory Function Decrements

Evidence from a prospective study and cross-sectional studies in a few U.S. populations of children indicates associations of higher blood Pb level with increases in hearing thresholds and decreases auditory processing or auditory evoked potentials with adjustment for potential confounding by SES in most studies and by child health and nutritional factors in some studies. The high participation rates, particularly in the prospective study with follow-up from birth, reduce the likelihood of biased participation by children with higher blood Pb levels. Across studies, associations were found with blood Pb levels measured at various time periods, including prenatal maternal, neonatal (10 day, mean 4.8 µg/dL), lifetime average (to age 5 years), and concurrent (ages 4-19 years) blood Pb levels (median 8 µg/dL). Evidence for Pb-associated increases in hearing thresholds or latencies of auditory evoked potentials was found in adult monkeys with lifetime dietary Pb exposure. However, these effects in adult animals were found with higher peak or concurrent blood Pb levels (i.e., 33-150 µg/dL) than those relevant to this ISA; thus, the biological plausibility for epidemiologic observations is unclear. The evidence in children, particularly that from a prospective study and observations of

decreased auditory evoked potentials in animals indicating a possible mode of action, but uncertainties related to effects on auditory function in juvenile animals with relevant Pb exposures, is sufficient to conclude that a causal relationship is likely to exist between Pb exposure and decrements in auditory function in children.

Visual Function Decrements

A study in children and a few studies in animals show Pb-associated increases in supernormal electroretinograms, the biological relevance of which is unclear. Because the available epidemiologic and toxicological evidence is of insufficient quantity, quality, and consistency, the evidence is inadequate to determine that a causal relationship exists between Pb exposure and visual function in children.

Motor Function Decrements

Evidence from prospective studies in a few populations indicates associations of decrements in fine and gross motor function with higher neonatal, concurrent, and lifetime average blood Pb levels in children ages 4.5-6 years and with higher earlier childhood (ages 0-5 year average, age 78 months) blood Pb levels in children ages 15-17 years. The means for these blood Pb metrics ranged from 4.8 to 12 µg/dL. These associations were found with adjustment for several potential confounding factors, including SES, parental caregiving quality, and child health and without indication of substantial selection bias. Evidence from cross-sectional studies was less consistent. The biological plausibility for associations observed in children is provided by a study that found poorer balance in male mice with relevant gestational to early postnatal (PND10) Pb exposures. The limited available evidence in a few prospective cohorts of children, but uncertainty because of the limited available findings in mice with relevant exposures is sufficient to conclude that a causal relationship is likely to exist between Pb exposure and decrements in motor function in children.

1.6.1.2 Adults

Cognitive Function

In adults without occupational exposure, recent prospective studies in the NAS and BMS cohorts indicate associations of higher baseline tibia (means 19, 20 µg/g) or patella (mean 25 µg/g) Pb levels with declines in cognitive function in adults (>age 50 years) over 2- to 4-year periods. While the specific covariates differed between studies, these bone

Pb-associated cognitive function decrements were found with adjustment for potential confounding factors such as age, education, SES, current alcohol use, and current smoking. Supporting evidence is provided by cross-sectional analyses of the NAS, BMS, and the Nurses' Health Study, which found stronger associations with bone Pb level than concurrent blood Pb level. Cross-sectional studies also considered more potential confounding factors, including dietary factors, physical activity, medication use, and comorbid conditions. The multiple exposures and health outcomes examined in many studies reduces the likelihood of biased participation specifically by adults with higher Pb exposure and lower cognitive function. The collective evidence indicates associations in cohorts of white men and women and a cohort of more ethnically diverse men and women. The specific timing, frequency, duration, and magnitude of Pb exposures contributing to the associations observed with bone Pb levels are not discernable from the evidence. Also, there is potential for residual confounding by age. The effects of recent Pb exposures on cognitive function decrements in adults were indicated in Pb-exposed workers by associations found with blood Pb levels, although these studies did not consider potential confounding by other workplace exposures. The biological plausibility for the effects of Pb exposure on cognitive function decrements in adults is provided by findings that relevant lifetime Pb exposures from gestation, birth, or after weaning induce learning impairments in adult animals and by evidence for the effects of Pb altering neurotransmitter function in hippocampus, prefrontal cortex, and nucleus accumbens. The associations between bone Pb level and cognitive function decrements consistently found in the few prospective and cross-sectional studies of adults without occupational Pb exposure, the coherence with animal findings, and toxicological evidence supporting modes of action but uncertainties related to potential residual confounding by age in epidemiologic studies are sufficient to conclude that a causal relationship is likely to exist between long-term cumulative Pb exposure and cognitive function decrements in adults.

Psychopathological Effects

Cross-sectional studies in a few populations demonstrate associations of higher concurrent blood or tibia Pb levels with self-reported symptoms of depression and anxiety in adults. The examination of multiple exposures and outcomes in the available studies does not provide strong indication of biased reporting of psychopathological effects specifically by adults with higher Pb exposures. In adults, Pb-associated increases in depression and anxiety were found with adjustment for age, SES, and in the NAS, daily alcohol intake. The biological plausibility for epidemiologic evidence is provided by observations of depression-like behavior in animals with dietary lactational Pb exposure, with some evidence at relevant blood Pb levels and by toxicological evidence supporting modes of action, including Pb-induced changes in the HPA axis and

dopaminergic and GABAergic systems. The associations of blood and bone Pb level with self-reported psychopathological effects found in the few studies of adults without occupational Pb exposure, the biological plausibility provided by the coherence with findings from a few animal studies and evidence for underlying modes of action, but uncertainties related to residual confounding of bone Pb results by age in epidemiologic studies are sufficient to conclude that a causal relationship is likely to exist between Pb exposure and psychopathological effects in adults.

Auditory Function Decrements

The the evidence provided by the analysis of NAS men for associations of higher tibia Pb level with a higher rate of elevations in hearing threshold over 20 years and observations of decreased auditory evoked potentials in animals indicating a possible mode of action but uncertainties related to effects on auditory function in adult animals with relevant Pb exposures, is suggestive of a causal relationship between Pb exposure and auditory function decrements in adults.

Visual Function Decrements

Studies in adult animals show differential effects on ERGs, depending on the timing and concentration of exposure. A case control study finds higher Pb in retinal tissue from macular degeneration cases but lacks rigorous statistical analysis and examination of potential confounding. Because the available epidemiologic and toxicological evidence is of insufficient quantity, quality, and consistency, the evidence is inadequate to determine that a causal relationship exists between Pb exposure and visual function in adults.

Neurodegenerative Disease

While evidence is inconclusive for Amyotrophic Lateral Sclerosis (ALS)and Alzheimer's disease, a few case-control studies each found higher blood Pb levels in adults with essential tremor and higher bone Pb levels in adults with Parkinson's disease. Because of the inconclusive evidence for some diseases and limitations such as reverse causation for essential tremor and the lack of consideration for potential confounding by manganese (Mn) exposure for both essential tremor and Parkinson's disease, the evidence is inadequate to determine that a causal relationship exists between Pb exposure and neurodegenerative diseases.

1.6.2 Cardiovascular Effects

The 2006 Pb AQCD (U.S. EPA, 2006b) concluded that there was a relationship between higher blood Pb and bone Pb and cardiovascular effects in adults, in particular increased BP and increased incidence of hypertension, and recent evidence strengthens this conclusion. For the evaluation of causal relationships with Pb exposure, evidence was grouped in categories using the U.S. Surgeon General's Report on Smoking as a guideline (CDC, 2004). In addition to hypertension (Section 4.4.7.1) these categories include subclinical atherosclerosis (Section 4.4.7.2), coronary heart disease (Section 4.4.7.3), and cerebrovascular disease (Section 4.4.7.4). Examination of measures of subclinical atherosclerosis provides the opportunity to assess the pathogenesis of vascular disease at an earlier stage. Studies that examine markers of subclinical atherosclerosis (such as PAD [i.e., ankle-brachial index]) and generalized atherosclerosis (i.e., IMT) are included in this category. Coronary heart disease (CHD) results from interruption of the blood supply to a part of the heart resulting from atherosclerosis of the coronary arteries, with acute injury and scarring leading to permanent damage to the heart muscles. A disrupted HRV has been associated with a higher mortality after MI and is used as a predictor of the physiological processes underlying CHD (Buccelletti et al., 2009). Studies that examine incidence of MI, IHD, HRV, and mortality from CHD, MI, or IHD are included in this category. Cerebrovascular disease describes a group of conditions involving the cerebral blood vessels that result in transient or permanent disruption of blood flow to the brain. These conditions include stroke, transient ischemic attack, and subarachnoid hemorrhage. Both hypertension and atherosclerosis are risk factors for cerebrovascular disease and the mechanisms for these outcomes also apply to cerebrovascular disease.

Hypertension

Prospective epidemiologic studies consistently find associations of blood and bone Pb levels with hypertension incidence and increased blood pressure (BP). These findings have been replicated across multiple high-quality studies comprising large and diverse populations. Further support is provided by multiple cross-sectional analyses. While the adjustment for specific factors varied by study, the collective body of epidemiologic evidence included adjustment for multiple potential key confounding factors, including age, diet, sex, body mass index (BMI), blood pressure lowering medication use, SES, race/ethnicity, alcohol consumption, cholesterol, smoking, pre-existing disease (i.e., diabetes), measures of renal function, and co-exposures (i.e., Cd) thus reducing the uncertainties related to confounding bias. Meta-analyses underscore the consistency and reproducibility of the Pb-associated increases in BP and hypertension (Navas-Acien et al., 2008; Nawrot et al., 2002). Nawrot et al. (2002) found that a doubling of concurrent blood Pb level (between 1 and >40 μg/dL) was associated with a 1 mmHg increase in systolic BP and a 0.6 mmHg increase in diastolic BP. Navas-Acien et al. (2008) found an association of higher bone Pb levels with increased BP in their pooled analysis. Although recent epidemiologic studies in adults report associations in populations with relatively low mean concurrent blood Pb levels, the majority of individuals in these adult populations were likely to have had higher levels of Pb exposure earlier in life. Thus, there is uncertainty concerning the specific Pb exposure level, timing, frequency, and duration contributing to the associations observed in the epidemiologic studies. A causal relationship of Pb exposure with hypertension is supported by evidence from experimental animal studies that demonstrate effects on BP after long-term Pb exposure resulting in mean blood Pb levels of greater than 10 μg/dL or greater (Figure 4-21). Further, there was no evidence of a threshold below which no significant association of blood Pb level with BP was observed among the NHANES II population, 20-74 years old between 1976 and 1980 with concurrent blood Pb levels ranging from 7-34 μg/dL (Schwartz, 1991). Relevant mode of action is demonstrated and coherence for the evidence of the effect of Pb exposure on BP and hypertension is further provided by epidemiologic evidence indicating associations with cardiovascular conditions related to increased BP including mortality, CHD, stroke, and cardiac failure. Thus, the overall evidence is sufficient to conclude that there is a causal relationship between Pb exposure and hypertension.

Subclinical Atherosclerosis

A limited number of studies have evaluated markers of subclinical atherosclerosis following Pb exposure in humans or animals. An NHANES analysis of adults (1999-2000) found an association between concurrent blood Pb and PAD that was robust to

adjustment for cadmium (Cd) and other potential confounding factors (Navas-Acien et al., 2004). A second more recent analysis with adjustment for numerous potential confounders reported an increasing trend in the odds of PAD across concurrent blood Pb level groups in adults within the NHANES population (Muntner et al., 2005). Evidence of plausible biological mechanisms (e.g., oxidative stress, inflammation, endothelial cell dysfunction) is clearly described in the animal toxicological literature but animal studies have provided only limited evidence to suggest Pb exposure may initiate atherosclerotic vessel disease. Further, the specific Pb exposure level, timing, frequency, and duration contributing to the observed association with PAD is not discernable from the epidemiologic evidence. Thus, the combined limited epidemiologic and toxicological evidence is suggestive of a causal relationship between Pb exposure and subclinical atherosclerosis.

Coronary Heart Disease

Prospective epidemiologic studies of cohorts of adults during the period 1976-1994 consistently report that blood Pb level is associated with risk of CHD-related mortality from cardiovascular disease, specifically MI, IHD, and HRV (Figure 4-29 and Table 4-23). Several recent studies also report associations between biomarkers of Pb and incidence of CHD-related outcomes including a prospective analysis reporting an increased incidence of IHD (physician confirmed MI, angina pectoris) with blood and bone Pb levels. In addition, Weisskopf et al. (2009) found that patella bone Pb levels were associated with increased mortality from IHD (similar magnitude non-statistically significant associations were observed with tibia Pb levels) among men enrolled in the NAS. The level, timing, frequency, and duration of Pb exposure contributing to CHD in adult populations with higher past than recent exposure is not discernable from the evidence. However, coherence for the associations in humans is supported by the observation of thrombus formation in animals after long term Pb exposure (Sections 4.4.7.3 and 4.4.3) and mode of action for Pb-induced CHD (i.e., hypertension, HRV, increased corrected QT (QTc) interval, and corrected QRS complex (QRSc) duration in electrocardiogram [ECG]) in humans and animals (Sections 4.4.2 and 4.4.3.4). The overall evidence is sufficient to conclude that there is a causal relationship between Pb exposure and coronary heart disease.

Cerebrovascular Disease

Both hypertension and atherosclerosis are risk factors for cerebrovascular disease and the mechanisms for these outcomes also apply to cerebrovascular disease. Despite strong evidence for hypertension and CHD and Pb exposure, very few epidemiologic or

toxicological studies have examined the effects of Pb exposure on cerebrovascular disease (Section 4.4.7). These epidemiologic studies provide limited evidence for increased risk of mortality from stroke and are insufficient evidence to inform the presence or absence of a causal relationship between cerebrovascular disease and Pb exposure. Thus, the current evidence is inadequate to determine that a causal relationship exists between Pb exposure and cerebrovascular disease.

1.6.3 Renal Effects

Recent epidemiologic and toxicological studies evaluated in the current review support and expand upon the strong body of evidence presented in the 2006 Pb AQCD (U.S. EPA, 2006b) indicating that Pb exposure is associated with reduced kidney function (Section 4.5.5). The causal determination for reduced kidney function is informed by evidence for reduced GFR, reduced creatinine clearance, and increased serum creatinine.

Reduced Kidney Function

There are multiple high quality epidemiologic studies and clear biological plausibility but uncertainty regarding the potential for reverse causality to explain findings in humans. Among epidemiologic studies, key evidence is provided by the few available longitudinal studies which better characterized the temporal sequence between Pb exposure and changes in renal function by showing associations between baseline bone or blood Pb levels and reduced kidney function over time in men in the Boston, MA area and greater progression of kidney disease in CKD patients. Evidence from longitudinal studies also addressed the potential for reverse causality by showing the persistence of blood Pb-associated decrements in kidney function in the range of normal kidney function and demonstrated stronger associations than cross-sectional analyses of the same data. Cross-sectional adult studies provide supportive evidence but are weighed less than the prospective studies in conclusions because by design they do not inform directionality (i.e., reverse causality). Inconsistencies were noted in occupational studies and studies of children, and important study design limitations were noted in clinical trials of chelation in CKD patients. These inconsistencies and limitations preclude strong inferences from the results of these three study groups. Longitudinal studies found Pb-associated decrements in renal function in populations with mean blood Pb levels of 7 and 9 µg/dL. However, the contributions of higher past Pb exposures cannot be excluded. Animal toxicological studies provide clear biological plausibility with evidence for Pb-induced kidney dysfunction at blood Pb levels greater than 30 µg/dL; however, evidence in animals with blood Pb levels < 20 µg/dL is generally not available. Studies with mean

blood Pb levels between 20 and 30 µg/dL provide some evidence for dysfunction in kidney function measures (e.g., decreased creatinine clearance, increased serum creatinine, increased BUN). Animal studies also provide biological plausibility for the associations observed between blood Pb levels and reduced kidney function with evidence for Pb-induced hypertension, renal oxidative stress and inflammation, morphological changes, and increased uric acid. Collectively, the evidence integrated across epidemiologic and toxicological studies with uncertainties related to the potential for reverse causation, is suggestive of a causal relationship between Pb exposures and reduced kidney function among adults.

1.6.4 Immune System Effects

The cumulative body of epidemiologic and toxicological evidence from the 2006 Pb AQCD (U.S. EPA, 2006b) and the current assessment describes several effects of Pb exposure on the immune system related to a shift from T-derived lymphocyte helper (Th)1 - to Th2 -type responses, including an increase in atopic and inflammatory conditions and a decrease in host resistance (Section 4.6.8). Outcomes related to an increase in atopic and inflammatory conditions (Section 4.6.8.1) include asthma, allergy, increased IgE, and mode of action endpoints such as selective differentiation of Th2 cells, increased production of Th2 cytokines, B cell activation, and inflammation. Outcomes related to decreased host resistance (Section 4.6.8.2) include enhanced susceptibility to bacterial and viral infection, suppressed delayed type hypersensitivity (DTH), and those describing mode of action, i.e., decreased production of Th1 cytokines, reduced phagocyte function, and increased inflammation. A small body of studies indicates the effects of Pb exposure on autoimmunity (Section 4.6.8.3).

Atopic and Inflammatory Conditions

Prospective studies in a few populations of children ages 1-5 years indicate associations of asthma and allergy with prenatal cord blood Pb levels or blood Pb levels measured sometime before the outcome, with a cross-sectional study providing supporting evidence with associations with concurrent blood Pb level. Prospective design, lack of selective participation of subjects, and objective assessment of outcomes reduce the likelihood of undue selection bias and reverse causation. These few studies varied in their consideration for potential confounding by SES and exposure to smoking or allergens. Thus, uncertainty remains regarding residual confounding in associations observed between blood Pb levels and asthma and allergy in children. The evidence for asthma and allergy is supported by cross-sectional associations found between higher concurrent

blood Pb levels in children and higher IgE, an important mediator of asthma and allergy. The biological plausibility for the effects of Pb on IgE is provided by consistent findings in animals with gestational or gestational-lactational Pb exposures, with some evidence at blood Pb levels relevant to humans. In epidemiologic studies, higher IgE and higher asthma prevalence were examined and found mostly in children with concurrent blood Pb levels >10 µg/dL. Coherence for the evidence of Pb-associated increases in asthma, allergy, and IgE is found with evidence for most of the examined endpoints related to mode of action, i.e., Pb-induced increases in Th2 cytokine production and inflammation in animals. Neither toxicological nor epidemiologic evidence clearly identifies an individual critical lifestage or duration of Pb exposure associated with atopic and inflammatory conditions but points to an influence of gestational and cumulative Pb exposures. The combined epidemiologic evidence in a few populations and toxicological evidence supporting a relationship between Pb exposure and asthma, allergy, and shift to a Th2 phenotype as an underlying mode action but some uncertainty regarding potential confounding is sufficient to conclude that a causal relationship is likely to exist between Pb exposures and an increase in atopic and inflammatory conditions.

Decreases in Host Resistance

Much of the evidence on decreased host resistance was available in the 2006 Pb AQCD (U.S. EPA, 2006b) and is summarized in Section 4.6.5.1 (and Section 4.6.8.2). Animal toxicological observations are the primary contributors to the evidence for Pb-induced decreased host resistance. Several studies in rodents show that dietary Pb exposure producing relevant blood Pb levels (7-25 µg/dL) results in increased susceptibility to bacterial infection and suppressed DTH. Further, coherence is found with evidence describing modes of action, including suppressed production of Th1 cytokines and decreased macrophage function in animals. These effects were found with gestational, lactational, adult-only, and lifetime Pb exposures of animals, without an individual critical lifestage of exposure identified. A few cross-sectional epidemiologic studies indicate Pb-associated increases in respiratory infections but limitations, including the lack of rigorous methodology and consideration for potential confounding produce uncertainty in the epidemiologic evidence for decreased host resistance in humans. The consistent toxicological evidence in animals for increased susceptibility to bacterial infection, suppressed DTH, and related modes of action but uncertainty in the epidemiologic evidence in humans is sufficient to conclude that a causal relationship is likely to exist between Pb exposure and decreased host resistance.

Autoimmunity

Toxicological evidence describes the potential of Pb to increase autoimmunity, with a few previous studies showing Pb-induced generation of auto-antibodies (Hudson et al., 2003; Bunn et al., 2000; El-Fawal et al., 1999; Waterman et al., 1994) and recent studies providing indirect evidence by showing formation of neoantigens that could result in the formation of auto-antibodies (Table 4-34). Several observations were made in animals injected with Pb, which is a route of exposure with less relevance to humans. Higher levels of auto-antibodies also were found in Pb-exposed battery workers; however, implications are limited because of the high blood Pb levels (range: 10-40 µg/dL) of some of the workers and lack of consideration for potential confounding by several factors, including other occupational exposures (El-Fawal et al., 1999). Because results from both available toxicological and epidemiologic studies do not sufficiently inform Pb-induced generation of auto-antibodies with relevant Pb exposures, the evidence is inadequate to determine if there is a causal relationship between Pb exposure and autoimmunity.

1.6.5 Hematological Effects

Recent toxicological and epidemiologic studies support evidence presented in in previous assessments including the 2006 Pb AQCD (U.S. EPA, 2006b) describes the effect of exposure to Pb on hematological outcomes such as RBC survival and function (Section 4.7.4.1) and altered heme synthesis (Section 4.7.4.2) . Endpoints considered within the category of RBC survival and function include alterations in multiple hematological parameters (e.g., Hb, Hct, MCV), oxidative stress (altered antioxidant enzyme activities, decreased cellular glutathione [GSH], and increased lipid peroxidation), increased cytotoxicity in RBC precursor cells, and mode of action endpoints such as decreased intracellular calcium concentrations $[Ca^{2+}]_i$, decreased adenosine-triphosphase (ATPase) activity, and increased phosphatidylserine expression. Endpoints related to altered heme synthesis include decreased activities of ALAD and ferrochelatase, and decreased levels of Hb.

Decreased Red Blood Cell Survival and Function

Experimental animal studies demonstrate that exposures via drinking water and gavage, resulting in blood Pb levels relevant to humans, alter several hematological parameters, increase measures of oxidative stress and increase cytotoxicity in red blood cell (RBC) precursor cells. Some of these effects have been observed in animal toxicological studies with exposures resulting in blood Pb levels 2-7 µg/dL. Support for these findings is

provided by biologically plausible modes of action including decreased intracellular calcium concentrations $[Ca^{2+}]_i$, decreased ATPase activity, and increased phosphatidylserine expression. Epidemiologic studies find associations in both adults and children of blood Pb levels with altered hematological endpoints, increased measures of oxidative stress, and altered hematopoiesis. Although the majority of these epidemiologic studies are limited by their lack of rigorous methodology, some studies in children did adjust for some potential confounding factors including age, sex, mouthing behavior, anemia, dairy product consumption, maternal age, education, employment, marital status, family structure, SES-related variables, strengthening their support for findings in experimental animals. Collectively, the strong evidence from toxicological studies that is supported by findings from mode of action and epidemiologic studies is sufficient to conclude that there is a causal relationship between Pb exposures and decreased RBC survival and function.

Heme Synthesis

Altered heme synthesis is demonstrated by a small, but consistent, body of studies in adult animals reporting that exposures via drinking water and gavage resulting in blood Pb levels relevant to humans (e.g., 6.5 µg/dL result in decreased ALAD and ferrochelatase activities. Supporting this evidence is a larger body of ecotoxicological studies that demonstrate decreased ALAD activity across a wide range of taxa exposed to Pb. Epidemiologic studies find associations in both adults and children between higher blood Pb levels and decreased ALAD and ferrochelatase activities. Although the majority of these studies are limited by their lack of rigorous methodology and consideration for potential confounding, some studies in children did adjust for or consider potential confounding factors (i.e. age, sex, urban/rural residence, height, weight, BMI), strengthening their support for the findings in the animal toxicological studies. Evidence for altered heme synthesis is also provided by a large body of toxicological and epidemiologic studies that report decreased Hb concentrations in association with Pb exposure or blood Pb levels. Collectively, the strong evidence from toxicological and ecotoxicological studies that is supported by findings from epidemiologic studies is sufficient to conclude that there is a causal relationship between Pb exposures and altered heme synthesis.

1.6.6 Reproductive and Developmental Effects

Many epidemiologic and toxicological studies of the effects of Pb on reproductive and developmental outcomes have been conducted since the 2006 Pb AQCD. The evaluation

of causal relationships with Pb exposure focuses on four areas: developmental effects (Section 4.8.5.1), birth outcomes (Section 4.8.5.2), male reproductive function (Section 4.8.5.3), and female reproductive function (Section 4.8.5.4).

Development

In cross-sectional epidemiologic studies of girls (ages 6-18 years) with mean and/or median concurrent blood Pb levels from 1.2-9.5 µg/dL consistent associations with delayed pubertal onset (measured by age at menarche, pubic hair development, and breast development) were observed. Although fewer studies were conducted in boys overall, associations between blood Pb levels and delayed puberty onset in boys (ages 8-15 years) were observed in cross-sectional and one longitudinal study (mean and/or median blood Pb levels 3-9.5 µg/dL). Potential confounders considered in the epidemiologic studies of both boys and girls that performed regression analyses varied across studies, with few studies considering confounding by nutritional factors. A limitation across most of the epidemiologic studies of blood Pb levels and delayed puberty is their cross-sectional design, which does not allow for an understanding of temporality. There is uncertainty with regard to the exposure frequency, timing, duration, and level that contributed to the associations observed in these studies. Experimental animal studies demonstrate that puberty onset in both males and females is delayed with Pb exposure. A recent animal study indicates that delayed pubertal onset may be one of the more sensitive developmental effects of Pb exposure with effects observed after gestational exposures leading to blood Pb levels in the female pup of 1.3-13 µg/dL. Experimental animal studies have also reported delayed male sexual maturity as measured with sex organ weight, among other outcomes, seeing significant decrements at blood Pb levels of 34 µg/dL. Findings from epidemiologic studies of the effect of Pb on postnatal growth are inconsistent and findings from the toxicological literature of the effect of Pb exposure are mixed with recent growth findings showing adult onset male obesity after gestational and lactational Pb exposure. Toxicological studies demonstrated effects of Pb exposure on other organ systems (effects on the eye, and alterations in the hematopoietic, hepatic systems and teeth.) The collective body of evidence integrated across epidemiologic and toxicological studies, based on the findings of delayed pubertal onset among males and females, is sufficient to conclude that there is a causal relationship between Pb exposure and developmental effects.

Birth Outcomes

Overall, epidemiologic studies of the association of various Pb exposure indicators with preterm birth report inconsistent findings. A recent epidemiologic study reported no

association between maternal blood Pb and neural tube defects. Some associations were observed between Pb and low birth weight when epidemiologic studies used measures of postpartum maternal bone Pb or air exposures. The associations were less consistent for maternal blood Pb measured during pregnancy or at delivery or umbilical cord and placenta Pb (maternal blood Pb or umbilical cord and placenta Pb were the biomarkers most commonly used in studies of low birth weight) but some associations between increased Pb biomarker levels and decreased low birth weight/fetal growth were observed. The effects of Pb exposure during gestation in animal toxicological studies included mixed findings with some studies showing reduction in litter size, implantation, and birth weight, and some showing no effect. Because some associations were observed in well-conducted epidemiologic studies of preterm birth and low birth weight/fetal growth, the evidence is suggestive of a causal relationship between Pb exposure and birth outcomes.

Male Reproductive Function

Key evidence is provided by toxicological studies in rodents, non-human primates, and rabbits showing detrimental effects on semen quality, sperm and fecundity/fertility with supporting evidence in epidemiologic studies of associations between blood Pb levels and detrimental effects on sperm. Pb exposures resulting in blood Pb levels from 5-43 µg/dL induced lower sperm quality and sperm production rate, sperm DNA damage, and histological or ultrastructural damage to the male reproductive organs. These effects were found in animals exposed to Pb during peripuberty or as adults for 1 week to 3 months. Pb exposure of male rats also was associated with subfecundity in female mates and lower fertilization of eggs in vitro. Detrimental effects of Pb on sperm were observed in epidemiologic studies with concurrent blood Pb levels of 25 µg/dL and greater among men occupationally exposed; however, these studies were limited because of their lack of consideration of potential confounding factors, including occupational exposures other than Pb. Additional epidemiologic studies among men with lower Pb biomarker levels were limited to infertility clinic studies that may lack generalizability; however, a well-conducted epidemiologic study that controlled for other metals as well as smoking reported a positive association with various detrimental effects in sperm (Telisman et al., 2007). The median concurrent blood Pb level in this study was 4.92 µg/dL. The specific timing, frequency, duration and level of Pb exposure associated with the blood Pb level and effects observed is not discernable from the epidemiologic evidence, however. Mode of action support is provided by several recent animal toxicological studies that showed that Pb induced oxidative stress within the male sex organs, increase apoptosis of spermatocytes and germ cells, and impaired germ cell structure and function. Based on the consistency and coherence of findings for the detrimental effects of Pb exposure on

sperm and semen in the toxicological literature, the support from epidemiologic studies, and biological plausibility provided by mode of action evidence, the evidence is sufficient to conclude that there is a causal relationship between Pb exposures and male reproductive function.

Female Reproductive Function

Epidemiologic and toxicological studies of reproductive function among females investigated whether Pb biomarker levels were associated with hormone levels, fertility, estrus cycle changes, and morphology or histology of female reproductive organs including the placenta (Section 4.8.5.4). Toxicological studies of experimental animals reported in the 2006 Pb AQCD (U.S. EPA, 2006b) demonstrated associations between Pb exposure and female reproductive function, although little evidence was provided by epidemiologic studies. Some studies have shown associations with concurrent blood Pb levels and altered hormone levels in adults, with inconsistency across studies may be due to the different hormones examined and the different timing in the menstrual and life cycles. There is some evidence of a potential relationship between Pb exposure and female fertility, but findings are also mixed. The majority of the epidemiologic studies are cross-sectional, and adjustment for potential confounders varies from study to study, with some potentially important confounders, such as BMI, not included in all studies. Also, most of the studies have small samples sizes and are generally of women attending infertility clinics. Animal toxicological studies that employ relevant prenatal or early postnatal Pb exposures observe that Pb contributes to placental pathology and inflammation, decreased ovarian antioxidant capacity, altered ovarian steroidogenesis and aberrant gestational hormone levels. Although epidemiologic and toxicological studies provide information on different exposure periods, both types of studies support the conclusion that Pb possibly affects at least some aspects of female reproductive function. Overall, the relationship observed with female reproductive outcomes, such as fertility, placental pathology, and hormone levels in some epidemiologic and toxicological studies, is sufficient to conclude that evidence is suggestive of a causal relationship between Pb exposure and female reproductive function.

1.6.7 Cancer

The toxicological literature provides the strong evidence for the effect of long-term exposure (i.e., 18 months or 2 years) to high concentrations of Pb (> 2,600 ppm) on cancer. The consistent evidence indicating Pb-induced carcinogenicity in animal models is substantiated by the mode of action findings from multiple high-quality toxicological

studies in animal and in vitro models from different laboratories. Based on such evidence, IARC has classified inorganic Pb compounds as a probable human carcinogen and the National Toxicology Program has listed Pb and Pb compounds as "reasonably anticipated to be human carcinogens." Strong evidence from animal toxicological studies demonstrates an association between Pb and cancer, genotoxicity or epigenetic modification. Carcinogenicity in animal toxicology studies with relevant routes of Pb exposure has been reported in the kidneys, testes, brain, adrenals, prostate, pituitary, and mammary gland, albeit at high doses of Pb. Epidemiologic studies of cancer incidence and mortality reported inconsistent results; one strong epidemiologic study demonstrated an association between blood Pb and increased cancer mortality, but the other studies reported weak or no associations. In the 2006 Pb AQCD, various indicators of Pb exposure were found to be associated with stomach cancer, and a recent study on stomach cancer and occupational Pb exposure reported mixed findings depending on the type of Pb exposure (organic Pb, inorganic Pb, or Pb from gasoline emissions). Similarly, some studies in the 2006 Pb AQCD reported associations between Pb exposure indicators and lung cancer. Recent epidemiologic studies of lung cancer focused on occupational exposures and reported inconsistent associations. The majority of epidemiologic studies of brain cancer had null results overall, but positive associations between Pb exposure indicators and brain cancer were observed among individuals with certain genotypes. Overall, the consistent and strong body of evidence from toxicological studies on carcinogenicity and potential modes of action but inconsistent epidemiologic evidence is sufficient to conclude that a causal relationship is likely to exist between Pb exposure and cancer.

1.7 Ecological Effects of Pb

Sections 1.7.1 and 1.7.2 are summaries of the evidence evaluated in Chapter 6 in which the effects of Pb on terrestrial and aquatic ecosystems are presented separately. The evidence supporting ecological causal determinations is synthesized across endpoints (reproduction, growth, survival, neurobehavioral effects, hematological effects, physiological stress) common to terrestrial, freshwater and saltwater biota in Section 1.7.3 (Table 1-3). An integration of the evidence across endpoints examined in both human health and ecological studies follows (Section 1.8). Consideration of atmospheric deposition of Pb as related to ecological effects is discussed under policy relevant considerations (Section 1.9.7).

1.7.1 Summary of Effects on Terrestrial Ecosystems

Historically, Pb poisoning is one of the earliest recognized toxicoses of terrestrial biota, occurring primarily through ingestion of spent shot by birds (Section 6.3.4.3). At the time of the 1977 Pb AQCD, few studies of Pb exposure and effects in wild animals other than birds were available. A limited number of rodent trapping studies and observations from grazing animals near smelters provided evidence for differences in Pb sensitivity among species and these findings were further supported in the 1986 and 2006 Pb AQCDs (U.S. EPA, 2006b, 1986b, 1977). Commonly observed effects of Pb on terrestrial organisms include decreased survival, reproduction, and growth, as well as effects on development, behavior, and ALAD activity (U.S. EPA, 2006b, 1986b, 1977).

In plants, Pb effects have been studied for several decades. At the time of the 1977 Pb AQCD, it was understood that Pb uptake in plants varied with species and with the size of the pool of Pb in the soil, and that most of the Pb taken up from the soil by plants other than trees remains in the roots, with translocation to other portions of the plant varying with species (U.S. EPA, 1977). Plant growth was recognized as an endpoint of Pb toxicity in plants in the 1977 Pb AQCD and additional effects of Pb on growth processes were reported in subsequent Pb AQCDs (U.S. EPA, 2006b, 1986b, 1977). In the 1977 Pb AQCD evidence for effects of Pb on forest-nutrient cycling and shifts in arthropod community composition was found in one study conducted in the vicinity of a smelting complex. In subsequent AQCDs, other ecosystem-level effects, including decreased species diversity, changes in floral and faunal community composition, and decreasing vigor of terrestrial vegetation have been reported near stationary sources of Pb (U.S. EPA, 2006b, 1986b, 1977; Watson et al., 1976).

Pb is either deposited directly onto plant surfaces, or onto soil where it can bind with organic matter or dissolve in pore water. The amount of Pb dissolved in soil pore water determines the impact of soil Pb on terrestrial ecosystems to a much greater extent than the total amount present. It has long been established that the amount of Pb dissolved in soil solution is controlled by at least six factors: (1) solubility equilibria; (2) adsorption-desorption relationship of total Pb with inorganic compounds; (3) adsorption-desorption reactions of dissolved Pb phases on soil organic matter; (4) pH; (5) cation exchange capacity (CEC); and (6) aging. Since 2006, further studies have contributed to the understanding of the role of pH, CEC, organic matter, and aging. Smolders et al. (2009) demonstrated that the two most important determinants of both Pb solubility and toxicity in soils are pH and CEC. However, they had previously shown that experimental aging, primarily in the form of initial leaching following addition of Pb, decreases soluble metal fraction by approximately one order of magnitude (Smolders et al., 2009). Since 2006, organic matter has been confirmed as an important influence on

Pb sequestration, leading to longer-term retention in soils with higher organic matter content, and also creating the potential for later release of deposited Pb. Aging, both under natural conditions and simulated through leaching, was shown to substantially decrease bioavailability to plants, microbes, and vertebrates.

Evidence over several decades of research, previously reviewed in Pb AQCDs and in more recent studies, shows that Pb accumulates in terrestrial plants, invertebrates and vertebrates. Studies with herbaceous plant species growing at various distances from smelters added to the existing strong evidence that atmospherically transported Pb is taken up by those plants. In most species tested, soil Pb taken up by the roots is not translocated into the stem and leaves. These studies did not establish the relative proportion that originated from atmospheric Pb deposited in the soil, as opposed to that taken up directly from the atmosphere through the leaves. In trees, studies have found that soil Pb generally is translocated to other parts, in contrast to herbaceous plants, and recent studies have shown that the proportion of Pb that is taken up through the leaves and trunk is likely substantial. One study attempted to quantify this proportion Pb that is taken up directly from the atmosphere suggested it amounts to 50% of the Pb contained in Scots pine (*Pinus sylvestris*) in Sweden (Klaminder et al., 2005).

Since the 2006 Pb AQCD, various species of terrestrial snails have been found to accumulate Pb from both diet and soil. Recent studies with earthworms have found that both internal concentration of Pb and mortality increase with decreasing soil pH and CEC, and the importance of the interaction of those factors with soil Pb has been strongly confirmed, but only very partially quantified. Tissue concentration differences have been found between species of earthworms that burrow in different soil layers, but it is unknown whether those tissue concentration differences are a direct result of species differences, a result of differences in soil variables such as in pH and CEC, or interactions among those factors. Because earthworms often sequester Pb in granules, some authors have suggested that earthworm Pb is not bioavailable to their predators. There is some evidence that earthworm activity increases Pb availability in soil, but it is inconsistent. In various arthropods collected at contaminated sites, recent studies found gradients in accumulated Pb that corresponded to gradients in soil with increasing distance from stationary sources.

There are a few recent studies of Pb bioavailability and uptake in birds since the 2006 Pb AQCD. Several found tissue levels in birds that indicated exposure to Pb, but none of the locations for these studies was in proximity to stationary anthropogenic sources, and the origin of the Pb could not be identified. A study at the Anaconda Smelter Superfund site found increasing Pb accumulation in gophers with increasing soil Pb around the location of capture. A study of swine fed various Pb-contaminated soils

showed that the form of Pb determined accumulation. Recent studies were able to measure Pb in the components of various food chains that included soil, plants, invertebrates, and vertebrates. They confirmed that trophic transfer of Pb is pervasive, but no consistent evidence of trophic magnification was found.

Evidence in this review further supports the findings of the previous Pb AQCDs that biological effects of Pb on terrestrial organisms vary with species and lifestage, duration of exposure, form of Pb, and soil characteristics. In photosynthetic organisms, experimental studies have added to the existing evidence of photosynthesis impairment in plants exposed to Pb, and have found damage to photosystem II due to alteration of chlorophyll structure, as well as decreases in chlorophyll content in diverse taxa, including lichens and mosses. Evidence of oxidative stress in response to Pb exposure has also been observed in plants. Reactive oxygen species were found to increase in broad bean and tomato plants exposed to increasing concentrations of soil Pb, and a concomitant increase in superoxide dismutase, glutathione, peroxidases, and lipid peroxidation, as well as decreases in catalase were observed in the same plants. Monocot, dicot, and bryophytic taxa grown in Pb-contaminated soil or in experimentally spiked soil all responded to increasing exposure with increased antioxidant activity. In addition, reduced growth was observed in some experiments, as well as genotoxicity, decreased germination, and pollen sterility.

In terrestrial invertebrates, evidence for Pb effects has included neurological and reproductive endpoints. Recently published studies have shown neuronal damage in nematodes exposed to concentrations of Pb [2.5 μM (0.5 mg Pb/L)] in laboratory settings, accompanied by behavioral abnormalities. Reproductive adverse effects were found at lower exposure in younger nematodes, and effects on longevity and fecundity were shown to persist for several generations. Increased mortality was found in earthworms, but was strongly dependent on soil characteristics including pH, CEC, and aging. Snails exposed to Pb through either topical application or through consumption of Pb-exposed plants had increased antioxidant activity, and decreased food consumption, growth, and shell thickness. Effects on arthropods exposed through soil or diet varied with species and exposure conditions, and included diminished growth and fecundity, endocrine and reproductive anomalies, and body malformations. Within each study, increasing concentration of Pb in the exposure medium generally resulted in increased effects, but the relationship between concentration and effects varied between studies, even when the same medium, e.g., soil, was used. Evidence suggested that aging and pH are important modifiers.

ALAD was identified in the 1977 Pb AQCD as a sensitive indicator of exposure to Pb in rats and waterfowl, and became regarded as a biomarker of exposure in many terrestrial

vertebrates. Other effects of Pb on vertebrates reviewed in Pb AQCDs and the current document include decreased white blood cell counts and behavioral anomalies observed in amphibians and reptiles. However, large differences in effects were observed at the same concentration of Pb in soil, depending on whether the soil was freshly amended or field-collected from contaminated areas. As in most studies where the comparison was made, effects were smaller when field-collected soils were used. In some birds, maternal elevated blood Pb level was associated in recent studies with decreased hatching success, smaller clutch size, high corticosteroid level, and abnormal behavior. Some species evidenced little or no effect of elevated blood Pb level. Effects of dietary exposure were studied in several mammalian species, and cognitive, endocrine, immunological, and growth effects were observed.

Recent evidence reviewed in Sections 6.3.6 and 6.3.12.7 demonstrates that exposure to Pb is generally associated with negative effects in terrestrial ecosystems. It also demonstrates that many factors, including species and various soil physiochemical properties, interact strongly with Pb concentration to modify those effects. In these ecosystems, where soil is generally the main component of the exposure route, Pb aging is a particularly important factor, and one that may be difficult to reproduce experimentally. Without quantitative characterization of those interactions, characterizations of exposure-response relationships would likely not be transferable outside of experimental settings. Since the 2006 Pb AQCD, few studies of exposure-response have been conducted, and results have been inconsistent. Table 6-4 summarizes studies of reproduction, growth, and survival in terrestrial organisms that have been published since 2006, and in which concentration-response data were reported.

Recent evidence of effects of Pb at the community and ecosystem levels of biological organization include several studies of the ameliorative effects of mycorrhizal fungi on plant growth in the presence of Pb, attributed to decreased uptake of Pb by plants, although both mycorrhizal fungus and plant were negatively affected at the exposures assessed. Most recently published research on community and ecosystem-level effects of Pb has focused on soil microbial communities, which have been shown to be impacted in both composition and activity. Many of the recent studies of effects on soil microbial communities have taken place in environments contaminated with multiple metals, and some have attempted to separate the effects of individual metals when possible. Soil microbial activity was generally diminished, but in some cases recovered over time. Species and genotype composition were consistently altered, and those changes were long-lasting or permanent. Recent studies have addressed differences in sensitivity between species explicitly, and have clearly demonstrated high variability between related species, as well as within larger taxonomic groupings. Mammalian no observed effect concentration (NOEC) values expressed as blood Pb levels were shown to vary by

a factor of 8, while avian blood NOECs varied by a factor of 50 (Buekers et al., 2009). Protective effects of dietary Ca^{2+} have been found in plants, birds, and invertebrates.

1.7.2 Summary of Effects on Aquatic Ecosystems

Effects of Pb on plants, invertebrates, and vertebrates are reported for both freshwater and saltwater ecosystems. Although effects of Pb exposure are likely mediated through common mode(s) of action across freshwater and marine/estuarine taxa, these ecosystems are considered separately because of different environmental and physiological factors that influence Pb toxicity such as bioavailability of the metal, form of Pb, water quality parameters and adaptations in freshwater and saltwater organisms. Toxicity of Pb also varies by organism, lifestage and duration of exposure. (U.S. EPA, 2006b, 1986a). Closely related organisms can vary greatly in bioaccumulation of Pb and other non-essential metals as well as in their susceptibility to Pb. Pb effects on aquatic biota were previously assessed in the 1977 Pb AQCD, the 1986 Pb AQCD and the 2006 Pb AQCD (U.S. EPA, 2006b, 1986a, 1977).

Exposure of freshwater and estuarine organisms to Pb, and associated effects, are tied to terrestrial systems via watershed processes (Section 6.2). Atmospherically-derived Pb can enter aquatic systems through runoff from terrestrial systems or via direct deposition over a water surface. In aquatic ecosystems affected by Pb, exposures are most likely characterized as low dose, chronic exposures. Once Pb enters surface waters, its solubility and subsequent bioavailability are influenced by Ca^{2+} concentration, pH, alkalinity, total suspended solids, and dissolved organic carbon (DOC), including humic acids. In saltwater, higher levels of ions additionally affect Pb bioavailability. In sediments, Pb bioavailability may be influenced by the presence of other metals, sulfides, iron (Fe-) and manganese (Mn-)oxides, and physical disturbance. Recent studies provide further evidence for the role of modifying factors such as pH, DOC, and hardness. Toxicity of the same concentration of Pb can vary greatly under different experimental conditions.

As recognized in the 2006 Pb AQCD and further supported in this review, uptake of Pb by aquatic invertebrates and vertebrates may preferentially occur via exposure routes other than direct absorption from the water column such as ingestion of contaminated food and water, uptake from sediment pore waters, or incidental ingestion of sediment (U.S. EPA, 2006b). Currently available models for predicting bioavailability focus on acute toxicity and do not consider all possible routes of uptake. They are therefore of limited applicability, especially when considering species-dependent differences in uptake and bioaccumulation of Pb. Recent evidence supports the 2006 Pb AQCD

conclusion that processes such as Pb adsorption, complexation, and chelation alter bioavailability to aquatic organisms.

Biological Effects of Pb on Freshwater Plants, Invertebrates and Vertebrates

Recent evidence further supports the findings of the previous Pb AQCDs that waterborne Pb is highly toxic to freshwater plants, invertebrates and vertebrates, with toxicity varying with species and lifestage, duration of exposure, form of Pb, and water quality characteristics. Concentration-response data from freshwater organisms indicate that there is a gradient of response to increasing Pb concentration and that some effects in sensitive species are observed at concentrations of Pb quantified in U.S. surface waters (Table 1-2).

The toxicity of Pb to aquatic algae and plants has been recognized in earlier EPA reviews of this metal. In the 1977 Pb AQCD, differences in sensitivity to Pb among different species of algae were reported and concentrations of Pb varied within and between genera. This observation was subsequently generalized across aquatic taxa (U.S. EPA, 1977). At the time of the 1977 Pb AQCD, the information available on effects of Pb on freshwater plants was limited. For plants in general, Pb was recognized to affect photosynthesis, mitosis, and growth, but at concentrations much higher than typically found in the environment. Effects of Pb on plants reported in subsequent Pb AQCDs included decreased growth, deformation of cells, and blocking of the pathways that lead to pigment synthesis, thus affecting photosynthesis.

Effects of Pb on aquatic plants supported by additional evidence in this review include oxidative damage, decreased photosynthesis, and reduced growth. Most recent studies report effects on growth at concentrations much higher than Pb typically encountered in the environment, however, some sublethal endpoints such as effects on chlorophyll were reported at concentrations in the 100 to 200 µg Pb/L range, albeit still much higher than those typically encountered in U.S. surface waters (Table 1-1). Elevated levels of antioxidant enzymes are commonly observed in aquatic plant, algae, and moss species exposed to Pb (U.S. EPA, 1977) and recent evidence continues to support this observation. Recent studies on uptake of Pb by aquatic plants support the findings of previous Pb AQCDs that all such plants with roots tend to sequester larger amounts of Pb in their roots than in their shoots, and provide additional evidence for species differences in compartmentalization of sequestered Pb and in responses to Pb in water and sediments. Exposure-response relationships in which increasing concentrations of Pb leads to increasing effects have consistently been reported in freshwater algae and macrophytes, suggesting that effects on growth and antioxidant activity are also occurring at lower

concentrations, however, most current observations of Pb effects in freshwater plants are at concentrations that exceed Pb concentration values available for U.S. surface waters (Table 1-1).

The largest body of evidence for effects of Pb at or near concentrations encountered in U.S. surface waters is from invertebrates. In the 1986 Pb AQCD (U.S. EPA, 1986a) and 2006 Pb AQCD (U.S. EPA, 2006b), reduced reproduction, growth, and survival were reported in various species of freshwater invertebrates. In the 2006 Pb AQCD, concentrations at which effects were observed in aquatic invertebrates ranged from 5 to 8,000 µg Pb/L. Recent evidence for effects of Pb on reproduction, growth, and survival supports findings in previous Pb AQCDs (Table 6-5). In a series of 48-hour acute toxicity tests using a variety of natural waters across North America, LC_{50} values ranged from 29 to 180 µg Pb/L tests with the cladoceran *Ceriodaphnia dubia* (Esbaugh et al., 2011). In this same species, increased DOC leads to an increased mean EC_{50} for reproduction as low as 25 µg Pb/L. Reproductive and growth effects have also been reported in rotifer, midge and mayfly species near the range of Pb concentrations encountered in freshwater habitats. Several studies in this review have provided evidence of growth effects at lower concentrations. Among the most sensitive species, growth of juvenile freshwater snails (*Lymnaea stagnalis*) was inhibited at an EC_{20} of <4 µg Pb/L (Grosell and Brix, 2009; Grosell et al., 2006b). A chronic value of 10 µg Pb/L, obtained in 28-day exposures of 2-month-old freshwater mussel (*Lampsilis siliquoidea*) juveniles, was the lowest genus-mean chronic value ever reported for Pb (Wang et al., 2010f).

Since the 2006 Pb AQCD, there is additional evidence for Pb effects on antioxidant enzymes, lipid peroxidation, stress response and osmoregulation in aquatic invertebrates, as well as additional information on Pb bioaccumulation. Recent studies using stable isotopes have enabled simultaneous measurement of uptake and elimination in several aquatic organisms to assess the relative importance of water versus dietary uptake. In uptake studies of various invertebrates, Pb was mainly found in the gills and digestive gland/hepatopancreas.

Pb effects on freshwater vertebrates were previously assessed in the 1977 Pb AQCD, the 1986 Pb AQCD and the 2006 Pb AQCD (U.S. EPA, 2006b, 1986a, 1977). Evidence of toxicity of Pb and other metals to freshwater organisms goes back to early observations of contamination of natural areas by Pb mining leading to extirpation of fish from streams (U.S. EPA, 1977). Recent evidence supports the findings of effects on survival, reproduction, and behavior reported in previous Pb AQCDs for freshwater vertebrates. In a series of 96-hour acute toxicity tests with fathead minnow conducted in a variety of natural waters across North America, LC_{50} values ranged from 41 to 3,598 µg Pb/L (Esbaugh et al., 2011). Reproductive effects associated with water quality parameters

were also noted with this species (Mager et al., 2010). In fish, several recent studies on behavioral effects of Pb indicate decreased prey capture rate, slower swim speed and decline in startle response and visual contrast with Pb exposure. These reported effects provide additional evidence for toxicity of Pb to fish. Chronic NOEC and EC_{10} values reported for trout, a sensitive species, are within the range of Pb occasionally encountered in U.S. surface waters (Table 6-2).

Observed responses of fish to Pb reported in the 1986 Pb AQCD and the 2006 Pb AQCD included inhibition of heme formation, alterations in brain receptors, effects on blood chemistry and hormonal systems, and decreases in some enzyme activities (U.S. EPA, 2006b, 1986a). Since the 2006 Pb AQCD, possible molecular targets for Pb neurotoxicity have been identified in fish and additional mechanisms of Pb toxicity have been elucidated in the fish gill and the fish renal system. In the 2006 Pb AQCD, amphibians were considered to be relatively tolerant to Pb. Observed responses to Pb exposure included decreased enzyme activity (e.g., ALAD reduction) and changes in behavior. Since the 2006 Pb AQCD, studies conducted at concentrations approaching environmental levels of Pb have indicated sublethal effects on tadpoles including deformities and decrements in growth and swimming ability.

In the 2006 Pb AQCD, adverse effects were found in freshwater fish at concentrations ranging from 10 to >5,400 μg Pb/L, generally depending on water quality variables (e.g., pH, hardness, salinity). Additional testing of Pb toxicity under conditions of varied alkalinity, DOC, and pH has been conducted since the last review. Effects in fish observed in recent studies fall within the range of concentrations observed in the previous Pb AQCD. Recent evidence also supports the 2006 conclusions that the gill is a major site of Pb uptake in fish, and that there are species differences in the rate of Pb accumulation and distribution of Pb within the organism. The anterior intestine has been newly identified as a site of uptake of Pb through dietary exposure studies. At the time of the publication of the 2006 Pb AQCD, trophic transfer of Pb through aquatic food chains was considered to be negligible. Measured concentrations of Pb in the tissues of aquatic organisms were generally higher in algae and benthic organisms than in consumers at higher trophic levels, indicating that Pb was bioconcentrated but not biomagnified. Some studies published since the 2006 Pb AQCD support the potential for transfer of Pb in aquatic food webs, while other studies indicate that Pb concentration decreases with increasing trophic level.

Ecosystem-level effects associated with Pb reported in previous Pb AQCDs include alteration of predator-prey dynamics, species richness, species composition, and biodiversity. Since the 2006 Pb AQCD, additional evidence for community and ecosystem level effects of Pb reviewed in Sections 6.4.7 and 6.4.12.7 have been observed

primarily in microcosm studies or field studies near contaminated areas (mining, effluent). Findings from field studies of aquatic communities in the vicinity of heavily contaminated sites include changes in species composition and species richness, predator/prey interactions, nutrient cycling and energy flow; however, Pb is often found coexisting with other metals and other stressors, which risk confounding the observed effects. Recent studies provide evidence in additional habitats for these community and ecological-level effects, specifically in aquatic macrophyte communities and sediment-associated communities. Different species may exhibit different responses to Pb-impacted ecosystems dependent not only upon other environmental factors (e.g., temperature, pH), but also on species sensitivity, lifestage, or seasonally-affected physiological state. Aquatic ecosystems with low pH and low dissolved organic matter are likely to be the most sensitive to the effects of atmospherically-deposited Pb.

Biological Effects of Pb on Saltwater Plants, Invertebrates and Vertebrates

In general, Pb toxicity to marine/estuarine plants, invertebrates and vertebrates is less well characterized than toxicity to Pb in freshwater systems due to an insufficient quantity of studies on saltwater organisms. In marine algae, effects on growth are observed in the most sensitive species at Pb concentrations that exceed amounts measured in the open sea or estuaries (Table 1-1). The majority of available studies of Pb effects on saltwater organisms are for invertebrate species. Evidence for Pb effects on reproduction, growth and survival as well as neurobehavioral, hematological and physiological stress endpoints are coherent with findings in freshwater invertebrates although most effects are observed at concentrations above 100 µg Pb/L which exceeds Pb typically encountered in seawater (Table 1-1). Fewer studies are available for Pb in marine sediments. In the amphipod, *Elasmopus laevis*, onset to reproduction was significantly delayed at 118 mg/Pb kg sediment; a concentration that the authors indicate is below the current marine sediment regulatory guideline for Pb (218 mg Pb/kg sediment) (Ringenary et al., 2007; NOAA, 1999). In the same study, no effects of Pb on adult survival in 28-day or 60-day sediment exposures were observed. Additional studies on reproduction, growth, and survival in marine invertebrates report effects above the range considered for causal determinations (Table II, Preamble). Several field monitoring studies with marine bivalves have used ALAD as a biomarker for Pb exposure and correlated ALAD inhibition to increased Pb tissue content. Field and laboratory studies provide evidence for antioxidant response to Pb exposure, however, most effects are observed at concentrations of Pb that are higher than concentrations detected in marine environments. No recent evidence for effects of Pb on marine vertebrates in controlled exposures was available for review.

1.7.3 Determinations of Causality for Effects on Ecosystems

Table 1-3 Summary of Pb causal determinations for plants, invertebrates, and vertebrates.

Level		Effect	Terrestrial[a]	Freshwater[a]	Saltwater[a]
Community- and Ecosystem-Level		Community and Ecosystem Effects (Sections 6.3.12.7, 6.4.12.7, and 6.4.21.7)	Likely Causal	Likely Causal	Inadequate
Population–Level Endpoints	**Organism–Level Responses**	Reproductive and Developmental Effects - Plants (Sections 6.3.12.1, 6.4.12.1, and 6.4.21.1)	Inadequate	Inadequate	Inadequate
		Reproductive and Developmental Effects - Invertebrates (Sections 6.3.12.1, 6.4.12.1, and 6.4.21.1)	Causal	Causal	Suggestive
		Reproductive and Developmental Effects - Vertebrates (Sections 6.3.12.1, 6.4.12.1, and 6.4.21.1)	Causal	Causal	Inadequate
		Growth - Plants (Sections 6.3.12.2, 6.4.12.2, and 6.4.21.2)	Causal	Likely Causal	Inadequate
		Growth - Invertebrates (Sections 6.3.12.2, 6.4.12.2, and 6.4.21.2)	Likely Causal	Causal	Inadequate
		Growth - Vertebrates (Sections 6.3.12.2, 6.4.12.2, and 6.4.21.2)	Inadequate	Inadequate	Inadequate
		Survival - Plants (Sections 6.3.12.3, 6.4.12.3, and 6.4.21.3)	Inadequate	Inadequate	Inadequate
		Survival - Invertebrates (Sections 6.3.12.3, 6.4.12.3, and 6.4.21.3)	Causal	Causal	Inadequate
		Survival - Vertebrates (Sections 6.3.12.3, 6.4.12.3, and 6.4.21.3)	Likely Causal	Causal	Inadequate
		Neurobehavioral Effects - Invertebrates (Sections 6.3.12.4, 6.4.12.4, and 6.4.21.4)	Likely Causal	Likely Causal	Inadequate
		Neurobehavioral Effects - Vertebrates (Sections 6.3.12.4, 6.4.12.4, and 6.4.21.4)	Likely Causal	Likely Causal	Inadequate
	Sub-organismal Responses	Hematological Effects - Invertebrates (Sections 6.3.12.5, 6.4.12.5, and 6.4.21.5)	Inadequate	Likely Causal	Suggestive
		Hematological Effects - Vertebrates (Sections 6.3.12.5, 6.4.12.5, and 6.4.21.5)	Causal	Causal	Inadequate
		Physiological Stress - Plants (Sections 6.3.12.6, 6.4.12.6, and 6.4.21.6)	Causal	Likely Causal	Inadequate
		Physiological Stress - Invertebrates (Sections 6.3.12.6, 6.4.12.6, and 6.4.21.6)	Likely Causal	Likely Causal	Suggestive
		Physiological Stress - Vertebrates (Sections 6.3.12.6, 6.4.12.6, and 6.4.21.6)	Likely Causal	Likely Causal	Inadequate

[a]Conclusions are based on the weight of evidence for causal determination in Table II of the ISA Preamble. Ecological effects observed at or near ambient Pb concentrations measured in soil, sediment and water in the most recent available studies (Table 1-1). were emphasized and studies generally within one to two orders of magnitude above the reported range of these values were considered in the body of evidence for terrestrial (Section 6.3.12), freshwater (Section 6.4.12) and saltwater (Section 6.4.21) ecosystems.

1.7.3.1 Effects on Development and Reproduction

Evidence from invertebrate and vertebrate studies from Pb AQCDs and in this review indicates that Pb is affecting reproductive performance in multiple species. Various endpoints have been measured in multiple taxa of terrestrial and aquatic organisms to assess the effect of Pb on development, fecundity, and hormone homeostasis, and they have demonstrated the presence of adverse effects. Reproductive effects are important when considering effects of Pb because impaired fecundity at the organism level of biological organization can result in a decline in abundance and/or extirpation of populations, decreased taxa richness, and decreased relative or absolute abundance at the community level (Suter et al., 2005; U.S. EPA, 2003a). The evidence is sufficient to conclude that there is a causal relationship between Pb exposures and developmental and reproductive effects in terrestrial (Section 6.3.12.1) and freshwater (Section 6.4.12.1) invertebrates and vertebrates. Although there is less evidence for reproductive and developmental effects of Pb in marine systems, available evidence is suggestive of a causal relationship between Pb exposure and reproductive and developmental effects in saltwater invertebrates (Section 6.4.21.1). The evidence is inadequate to conclude that there is a causal relationship between Pb exposures and developmental and reproductive effects in saltwater vertebrates and in either terrestrial or aquatic plants.

Recent evidence for developmental and reproductive endpoints in terrestrial invertebrates shown to be affected by Pb include hatching success in collembolans, increased development time in fruit flies and aphids, and disrupted hormone homeostasis in moths. These studies have generally used Pb concentrations that exceed Pb soil concentrations found at most U.S. locations (Table 1-1), but many of them included multiple concentrations, and responses increased with increasing concentration. In terrestrial vertebrates, recent evidence for decreased sperm count and quality in deer at a location contaminated by mining, and for decreased testis weight in lizards, support previous associations between Pb exposure and reproductive and developmental effects. Few studies are available that specifically address reproductive effects of Pb exposure in either terrestrial or aquatic plants.

In terrestrial invertebrates, Pb can alter developmental timing, hatching success, sperm morphology, and hormone homeostasis. In fruit flies, Pb exposure increased time to pupation and decreased pre-adult development. Sperm morphology was altered in earthworms exposed to Pb-contaminated soils. Pb may also disrupt hormonal homeostasis in invertebrates as studies with moths have suggested. Evidence of

multi-generational toxicity of Pb is also present in terrestrial invertebrates, specifically springtails, mosquitoes, carabid beetles, and nematodes where decreased fecundity in progeny of Pb-exposed individuals was observed. However, effects have only been studied in a small number of species.

For freshwater invertebrates, exposure to Pb under controlled conditions has provided evidence for reproductive effects on sensitive taxa (gastropods, amphipods, cladocerans) at or near the range of Pb concentration values available for U.S. surface waters (Table 1-1). Reproductive effects were reported to begin at 19 µg Pb/L for the snail *Lymnaea palustris* and 27 µg Pb/L for Daphnia sp. as reported in the 1986 Pb AQCD (U.S. EPA, 1986b). In a 42-day chronic study reviewed in the 2006 Pb AQCD, the LOEC for reproduction was 3.5 µg Pb/L in amphipods receiving both waterborne and dietary Pb (Besser et al., 2005). Several recent studies in snails, rotifers and other freshwater invertebrates support previous findings of adverse impacts to reproduction (Table 6-5) . Reproductive effects have also been observed in multi-generational studies with aquatic invertebrates. Larval settlement rate and rate of population increase was decreased in rotifers and marine amphipods. Rotifers have a reduced fertilization rate associated with Pb exposure that appears to be due to decreased viability of sperm and eggs.

In freshwater vertebrates there is evidence for reproductive and developmental effects of Pb. Recent evidence in frogs and freshwater fish continues to support developmental and reproductive effects of Pb in aquatic vertebrates reported in earlier Pb AQCDs. Pb-exposure in tadpoles has been demonstrated to delay metamorphosis, decrease larval size, produce subtle skeletal malformations, and to result in slower swimming speed. Previous Pb AQCDs have reported developmental effects in fish, specifically spinal deformities in larvae at a concentration of 120 µg Pb/L. In the 2006 Pb AQCD, decreased spermatocyte development in rainbow trout was observed at 10 µg Pb/L and in fathead minnow testicular damage occurred at 500 µg Pb/L. In more recent studies, reproduction in fathead minnows was affected in breeding exposures following 300-day chronic toxicity testing. However, reproductive performance was unaffected in zebrafish *Danio rerio* exposed to Pb-contaminated prey. In fish, there is recent evidence of Pb effects on steroid profiles from nominal exposure studies.

In terrestrial vertebrates, evidence from Pb AQCDs and more recent evidence support an association between Pb exposure and observed adverse reproductive effects. In mammals, few studies in the field have addressed Pb specifically: most available data in wild or grazing animals are from near smelters, where animals are co-exposed to other metals. Evidence obtained using mammals in the context of human health research demonstrates adverse effects of Pb on sperm, and on onset of puberty in males and females (Chapter 4), which is coherent with the partial evidence from mammals in the wild. Other

reproductive endpoints including spontaneous abortions, pre-term birth, embryo development, placental development, low birth weight, subfecundity, hormonal changes, and teratology were also affected, but less consistently. Recent toxicological data from animal studies support trans-generational effects, a finding that is also an area of emerging interest in ecology.

Many studies of effects on reproductive and developmental endpoints in terrestrial invertebrates and vertebrates have been conducted with soil Pb concentrations exceeding those found in most U.S. locations. Recent and past studies that include multiple, increasing concentrations of Pb, from background level to levels greater than those associated with heavily contaminated sites, showed exposure-dependent responses. For some sensitive aquatic species, recent evidence supports previous findings of reproductive and developmental effects of Pb and differential lifestage response at or near concentrations of Pb reported from natural environments.

1.7.3.2 Effects on Growth

Alterations in the growth of an organism can impact population, community and ecosystem level variables. Evidence is sufficient to conclude that there is a causal relationship between Pb exposures and effects on growth in terrestrial plants (Section 6.3.12.2) and freshwater invertebrates (Section 6.4.12.2). Evidence is sufficient to conclude that a causal relationship is likely to exist between Pb exposure and effects on growth in terrestrial invertebrates and freshwater plants. Evidence is inadequate to establish a causal relationship between Pb exposures and effects on growth in terrestrial and aquatic vertebrates and saltwater biota (Section 6.4.21.2).

Evidence for effects of Pb on growth is strongest in terrestrial plants. In invertebrates, evidence for effects of Pb on growth has been observed most extensively in freshwater taxa, with inhibition in sensitive species occurring in or near the range of Pb concentration values found in surveys of U.S. surface waters (Table 1-1). Vertebrates, particularly terrestrial, have been the object of a comparatively much smaller number of studies of the effects of Pb on growth. Growth effects observed in both invertebrates and vertebrates, however, underscore the importance of lifestage to overall Pb susceptibility. In general, juvenile organisms are more sensitive than adults. Evidence for growth effects of Pb in freshwater and terrestrial plant species is primarily supported by earlier Pb AQCDs. In aquatic invertebrates, the weight of the evidence continues to support growth effects of Pb with several recent studies reporting effects at ≤ 10 µg Pb/L, specifically in snails and mussels (Table 6-5). Also, growth effects in frogs are reported at lower concentrations in the current document than in earlier reviews.

There is evidence over several decades of research that Pb inhibits photosynthesis and respiration in plants, both of which reduce growth (U.S. EPA, 2006b, 1977). Many toxicity studies conducted in laboratory and greenhouse settings have reported effects on plants. These effects are typically observed in laboratory studies with high exposure concentrations or in field studies near stationary sources and heavily contaminated sites, but studies that include multiple concentrations of Pb show increased response with increasing concentration. Pb has been shown to affect photosystem II, altering the pigment structure, and decreasing the efficiency of visible light absorption by affected plants. Decreases in chlorophyll *a* and *b* content have been observed in various algal and plant species. Most primary producers experience EC_{50} values for growth in the range of 1,000 to 100,000 µg Pb/L with minimal inhibition of growth observed as low as 100 µg Pb/L (U.S. EPA, 2006b).

Growth effects of Pb on aquatic invertebrates are reviewed in the draft *Ambient Aquatic Life Water Quality Criteria for Lead* (U.S. EPA, 2008b) and the 2006 Pb AQCD (U.S. EPA, 2006b). In the 2006 Pb AQCD, the LOEC for growth of freshwater amphipods *Hyalella azteca* in 42-day chronic exposure to Pb was 16 µg Pb/L (Besser et al., 2005). Recent studies summarized in Table 6-5 provide additional evidence for effects on growth of aquatic invertebrates, with some effects observed at ≤ 10 µg Pb/L. Growth of juvenile freshwater snails *L. stagnalis* was inhibited below the lowest concentration tested resulting in an EC_{20} <4 µg Pb/L (Grosell and Brix, 2009; Grosell et al., 2006b). In the same study, the NOEC was 12 µg Pb/L and the LOEC was 16 µg Pb/L. The authors indicated that the observed effect level for Pb was very close to the current U.S. EPA water quality criteria for Pb (3.3 µg Pb/L normalized to test water hardness) (Grosell and Brix, 2009). In the freshwater mussel, fatmucket (*L. siliquoidea*) juveniles were the most sensitive lifestage (Wang et al., 2010f). A chronic value of 10 µg Pb/L in a 28-day exposure of 2-month-old fatmucket juveniles was the lowest genus mean chronic value ever reported for Pb. Growth effects are also reported in marine invertebrates at higher concentrations of Pb than sensitive freshwater invertebrates.

In Pb AQCDs, a few studies have reported growth effects of Pb on vertebrates including fish (growth inhibition), birds (changes in juvenile weight gain), and frogs (delayed metamorphosis, smaller larvae). A recent study reviewed in this ISA supports findings of growth effects in frogs and suggests that these effects may be occurring at lower concentrations than previously reported: the growth rate of tadpoles of the northern leopard frog exposed nominally to 100 µg Pb/L from embryo to metamorphosis was slower than the growth rate of the controls (Chen et al., 2006b). In this study, Pb concentrations in the tissues of tadpoles were quantified and the authors reported that they were within the range of reported tissue concentrations reported in wild-caught populations. Reports of Pb-associated growth effects in fish are inconsistent.

1.7.3.3 Effects on Survival

Survival is a biologically important response that may have a direct impact on population size and can lead to effects at the community and ecosystem level of biological organization. The evidence is sufficient to conclude that there is a causal relationship between Pb exposures and survival in terrestrial invertebrates (Section 6.3.12.3) and freshwater invertebrates and vertebrates (Section 6.4.12.3). Evidence is sufficient to conclude that a causal relationship is likely to exist between Pb exposure and survival in terrestrial vertebrates (Section 6.3.12.3). The evidence is inadequate to conclude that there is a causal relationship between Pb exposure and survival in terrestrial and freshwater plants (Section 6.3.12.3, and Section 6.4.12.3), as well as in all saltwater biota (Section 6.4.21.3). There is evidence for mortality in saltwater organisms at concentrations that greatly exceed Pb concentrations typically encountered in seawater. In general, marine organisms are less sensitive to Pb than freshwater species.

In terrestrial vertebrates, evidence for Pb effects on survival is primarily supported by Pb AQCDs with no recent studies reporting effects on survival at lower concentrations. For aquatic invertebrates recent studies support previous associations between Pb exposure and mortality at concentrations near the range of Pb concentration values available for U.S. surface waters (Table 1-1) in cladocerans, amphipods, and rotifers (Table 6-5). In aquatic vertebrates, there is recent evidence for effects in fish at <100 µg Pb/L. Pb is generally not phytotoxic to freshwater or terrestrial plants at concentrations found in the environment away from stationary sources and heavily contaminated sites, probably due to the fact that plants often sequester large amounts of Pb in roots, and that translocation to other parts of the plant is limited.

The relationship between Pb exposure and decreased survival rate has been well demonstrated in terrestrial and aquatic species, as presented in Sections 6.3.12.3, 6.4.12.3, 6.4.21.3, and in previous Pb AQCDs. Toxicological studies have established LC_{50} values for some species of plants, invertebrates, and vertebrates. From the LC_{50} data on Pb in this review and previous Pb AQCDs, a wide range of sensitivity to Pb is evident across taxa. LC_{50} values are usually much higher than Pb concentrations near contaminated sites, although physiological dysfunction that adversely impacts the fitness of an organism often occurs well below concentrations that result in mortality.

Freshwater aquatic invertebrates are generally more sensitive to Pb exposure than other taxa, with survival adversely impacted in a few species in laboratory studies at concentrations near typical ambient levels. Freshwater biota that exhibit sensitivity to Pb in the upper range of Pb concentrations measured in U.S. waters [median 0.50 µg Pb/L, range 0.04 to 30 µg Pb/L (U.S. EPA, 2006b)], include some species of gastropods, amphipods, cladocerans, and rotifers although the toxicity of Pb is highly dependent upon

water quality variables such as DOC, hardness, and pH. For example, amphipods tested under various water conditions exhibited sensitivity to Pb at <10 µg Pb/L (U.S. EPA, 2006c) and the present document). These impacted species may include endangered species or candidates for the endangered species list, such as the freshwater mussel *Lampsilis rafinesqueana* (Neosho mucket). The EC_{50} for foot movement (a measure of viability) for newly transformed juveniles of this species was 188 µg Pb/L. Other aquatic invertebrates such as odonates may be tolerant of Pb concentrations that greatly exceed Pb detected in aquatic ecosystems.

Terrestrial invertebrates typically tolerate high concentrations of Pb relative to concentrations found in most uncontaminated soils. In the 1986 Pb AQCD it was reported that Pb at environmental concentrations occasionally found near roadsides and smelters (10,000 to 40,000 µg Pb/g dry weight [mg Pb/kg]) can eliminate populations of bacteria and fungi on leaf surfaces and in soil. LC_{50} values for soil nematodes vary from 10-1,550 mg Pb/kg dry weight dependent upon soil organic matter content and soil pH (U.S. EPA, 2006b). In earthworms, 14 and 28 day LC_{50} values typically fall in the range of 2,400-5,800 mg Pb/kg depending upon the species tested.

Data on mortality of saltwater species associated with exposure to Pb is limited; however, in general, marine organisms are less sensitive to this metal than freshwater organisms and the highest toxicity is observed in juveniles. In one study, effects of Pb on survival at environmentally relevant concentrations of Pb in diet have been demonstrated through a simulated marine food chain in which the primary producer, the microalgae *Tetraselmis suecica*, was exposed nominally to 20 µg Pb/L and subsequently fed to brine shrimp *Artemia franciscana*, (mean Pb content 12 to 15 µg Pb/g) which were consumed by white-leg shrimp *Litopenaeus vannamei*, itself consumed by grunt fish *Haemulon scudderi* representing the top of the marine food chain (Soto-Jiménez et al., 2011b). Survival of brine shrimp was 25 to 35% lower than the control and both white shrimp and grunt fish had significantly higher mortalities than controls.

In vertebrates, toxicity is observed in terrestrial avian and mammalian species in laboratory studies over a wide range of doses (<1 to >1,000 mg Pb/kg body weight per day) as reviewed for the development of ecological soil screening levels (Eco-SSLs) (U.S. EPA, 2005b). The NOAELs for survival ranged from 3.5 to 3,200 mg Pb/kg per day. In freshwater vertebrates there is considerable historic information on Pb toxicity to fish. Recent studies support earlier AQCD findings of Pb effects on fish survival and indicate effects at lower concentrations when testing with juvenile lifestages. In a series of 96-hour acute toxicity tests with fathead minnow conducted in a variety of natural waters across North America, LC_{50} values ranged from 41 to 3,598 µg Pb/L and no Pb toxicity occurred in three highly alkaline waters (Esbaugh et al., 2011). Thirty day

LC_{50} values for larval fathead minnows ranged from 39 to 1,903 µg Pb/L in varying concentrations of DOC and Ca^{2+} (as $CaSO_4$) (Grosell et al., 2006a). In a recent study of rainbow trout fry at 2-4 weeks post-swim up, the 96-hour LC_{50} was 120 µg Pb/L at a hardness of 29 mg/L as calcium carbonate ($CaCO_3$), a value much lower than in testing with older fish (Mebane et al., 2008). In the same study, two chronic (>60 day) tests were conducted with rainbow trout and the LC_{20} values for survival were 34 µg Pb/L in the 69 day test and 113 µg Pb/L in the 62 day test.

1.7.3.4 Neurobehavioral Effects

Overall, the evidence from terrestrial and freshwater systems is sufficient to conclude that a causal relationship is likely to exist between Pb exposures and neurobehavioral effects in invertebrates and vertebrates (Sections 6.3.12.4 and 6.4.12.4). Evidence is inadequate to conclude that there is a causal relationship between Pb exposure and neurobehavioral endpoints in saltwater species (Section 6.4.21.4).

Observations from laboratory studies reported in Chapter 6 and previous Pb AQCDs have shown adverse effects of Pb on neurological endpoints in both terrestrial and freshwater animal taxa. Studies that consider mode-of-action and molecular targets of Pb toxicity in biota are now available for a few species. Recent studies have continued adding to the evidence from both invertebrate and vertebrate studies that Pb adversely affects behaviors such as food consumption, avoidance, and escape from predators, behavioral thermoregulation, and prey capture. These changes are likely to decrease the overall fitness of the organism. Recent evidence includes reports of behavioral responses across a larger variety of organisms including fish larvae born from Pb-exposed adults and reptiles, while some impairments in feeding and escaping behaviors were reported for the first time.

Central nervous system effects in fish recognized in previous Pb AQCDs include effects on spinal neurons and brain receptors. Recent evidence from this review identifies possible molecular targets for Pb neurotoxicity in fish. Additionally, there is recent evidence for neurotoxic action of Pb in invertebrates with exposure to Pb observed to cause changes in the morphology of gamma aminobutyric acid (GABA)-motor neurons in nematodes (*Caenorhabditis elegans*) (Du and Wang, 2009).

Decreased food consumption of Pb-contaminated diet has been demonstrated in some invertebrates (snails) and vertebrates (lizards, pigs, fish). Behavioral effects in grunt fish *H. scudderi*, occupying the top level of a simulated marine food chain included lethargy and decreased food intake in a 42-day feeding study (Soto-Jiménez et al., 2011b). These fish were fed white shrimp exposed to Pb via brine shrimp that were initially fed

microalgae cultured nominally at 20 µg Pb/L. In the same study, surfacing, reduction of motility, and erratic swimming were observed in the white shrimp after 30 days of exposure to Pb via diet. Pb may also decrease the ability of an organism to capture prey or escape predation. For example, Pb exposure has been demonstrated to adversely affect prey capture ability of certain fungal and fish species, and the motility of nematodes was adversely affected in Pb-contaminated soils (Wang and Xing, 2008). Prey capture ability was decreased in 10-day-old fathead minnows born from adult fish exposed to 120 µg Pb/L for 300 days, then subsequently tested in a 21-day breeding assay (Mager et al., 2010). Altered pattern of escape swimming in larval zebrafish exposed to Pb as embryos was reported at low nominal concentrations of Pb (2 and 6 µg Pb/L). Other behavioral effects of Pb observed in fish include increased hyperactivity and decreased ability to detect visual contrast. In a laboratory study, Pb-exposed gull chicks exhibited abnormal behaviors such as decreased walking, erratic behavioral thermoregulation and food begging that could make them more vulnerable in the wild (Burger and Gochfeld, 2005). The chicks were exposed to Pb via injection to produce feather Pb concentration approximately equivalent to those observed in wild gulls. Lizards exposed to Pb through diet in the laboratory exhibited abnormal coloration and posturing behaviors. These findings show strong coherence with findings from studies in laboratory animals that show that Pb induces changes in learning and memory (Section 4.3.2.3), as well as attention (Section 4.3.3) and motor function (Section 4.3.7).

1.7.3.5 Hematological Effects

Based on observations in both terrestrial and freshwater organisms and additionally supported by toxicological and epidemiological findings in laboratory animals and humans, evidence is sufficient to conclude that there is a causal relationship between Pb exposures and hematological effects in terrestrial and aquatic vertebrates (Sections 6.3.12.5 and 6.4.12.5). The evidence is sufficient to conclude that a causal relationship is likely to exist between Pb exposures and hematological effects in freshwater invertebrates and inadequate to conclude that there is a causal relationship between Pb exposures and hematological effects in terrestrial invertebrates. Limited evidence from marine invertebrates is suggestive of a causal relationship between Pb exposures and hematological effects (Section 6.4.21.5). The mode of action of Pb on ALAD activity is likely mediated through a common pathway in terrestrial, freshwater and saltwater organisms.

Recent studies add support to the strong body of evidence presented in Pb AQCDs that Pb exposure is associated with hematological responses in terrestrial and aquatic vertebrates. Lower ALAD activity has been significantly correlated with elevated blood

Pb levels in fish and mammals. In the 1986 Pb AQCD, decreases in RBC ALAD activity following Pb exposure were well documented in birds and mammals (U.S. EPA, 1986a). The draft *Ambient Aquatic Life Water Quality Criteria for Lead* summarized several studies of ALAD activity in fish (U.S. EPA, 2008b). Further evidence from the 2006 Pb AQCD and this review suggests that this enzyme is an indicator for Pb exposure across a wide range of taxa. Since the 2006 Pb AQCD, evidence of Pb effects on ALAD activity has been found in additional species of invertebrates and fish, and has been identified in bacteria. ALAD activity has been shown to vary greatly between species. In addition to consideration of ALAD activity, there is recent evidence for deceased white blood cell counts in amphibians affected by Pb exposure. The consistency and coherence of these findings of effects on ALAD activity in vertebrates are also supported by some evidence of Pb-induced alterations of blood chemistry in fish reported in the 2006 Pb AQCD (U.S. EPA, 2006b). This evidence is strongly coherent with observations from human epidemiologic and animal toxicology studies where a causal relationship was identified between Pb exposure and decreased RBC survival and function, and altered heme synthesis in humans and laboratory animals (Sections 1.6.5 and 4.7).

In environmental assessments of metal-impacted habitats, ALAD is a recognized biomarker of Pb exposure in invertebrates as well as vertebrates (U.S. EPA, 2006b). Recent field studies of ALAD activity include observations in songbirds and owls near historical mining areas and in bivalves collected from freshwater and estuarine environments. Evidence for hematological effects of Pb in saltwater invertebrates is limited primarily to field monitoring studies with bivalves.

1.7.3.6 Effects on Physiological Stress

Evidence is sufficient to conclude that there is a causal relationship between Pb exposures and physiological stress in terrestrial plants (Section 6.3.12.6). Evidence is sufficient to conclude that a causal relationship is likely to exist between Pb exposures and physiological stress in terrestrial invertebrates and vertebrates (Section 6.3.12.6) as well as freshwater plants, invertebrates and vertebrates (Section 6.4.12.6). Further evidence in saltwater invertebrates is suggestive of a causal relationship between Pb exposures and physiological stress (Section 6.4.21.6). Evidence is inadequate to conclude that there is a causal relationship between Pb exposure and physiological stress responses in saltwater plants and vertebrates.

Endpoints associated with physiological stress received no consideration prior to the 2006 Pb AQCD. Studies reviewed in the 2006 Pb AQCD reported stress-related effects including upregulation of antioxidant enzymes and increased lipid peroxidation (U.S.

EPA, 2006b). Recent evidence in additional species of terrestrial and freshwater plants, invertebrates and vertebrates support, and expand upon findings in the previous Pb AQCD. Some of these studies report findings within one to two orders of magnitude of the range of Pb concentrations measured in terrestrial and freshwater environments (Table 1-1). Recent studies include evidence for production of reactive oxygen species in terrestrial plant species and in freshwater algae and fish in response to Pb exposure.

In the current document and the 2006 Pb AQCD (U.S. EPA, 2006b), there is strong evidence of upregulation of antioxidant enzymes and increased lipid peroxidation associated with Pb exposure in many species of plants, invertebrates and vertebrates. In plants, increases of antioxidant enzymes with Pb exposure occur in algae, aquatic mosses, floating and rooted aquatic macrophytes, and terrestrial species. Most observations of antioxidant responses in plants typically occur at concentrations of Pb higher than found in the environment. However, in a few terrestrial plant species, increases of antioxidant enzymes occur at concentrations approaching the average Pb concentrations in U.S. soils (Table 1-1) and limited transplantation studies with aquatic plants indicate elevated antioxidant enzyme activity associated with Pb levels measured in sediments at polluted sites. There is evidence for antioxidant activity in invertebrates, including gastropods, mussels, and crustaceans, in response to Pb exposure. Some recent evidence for invertebrate antioxidant responses in freshwater bivalves, and marine bivalve and crustacean species indicates effects at Pb concentrations associated with polluted sites. Markers of oxidative damage are also observed in fish, amphibians, and mammals, both in the laboratory and in exposed natural environments. Across all biota, there are differences in the induction of antioxidant enzymes that appear to be species-dependent.

Additional stress responses observed in terrestrial and freshwater invertebrates include elevated heat shock proteins, osmotic stress and decreased glycogen levels. Heat shock protein induction by Pb exposure has been observed in zebra mussels and mites. Tissue volume regulation is adversely affected in freshwater crabs and glycogen levels decreased in freshwater snails following Pb exposure. Although correlated with Pb exposure, these responses are non-specific and may be altered with exposure to any number of environmental stressors.

Upregulation of antioxidant enzymes and increased lipid peroxidation are considered to be reliable biomarkers of stress, and suggest that Pb exposure induces a stress response in those organisms, which may increase susceptibility to other stressors and reduce individual fitness. The oxidative stress responses associated with Pb exposure are consistent in terrestrial biota and in freshwater organisms. Furthermore, these responses are also observed in experimental animal studies.

1.7.3.7 Community and Ecosystem Effects

More evidence for Pb toxicity to terrestrial and aquatic biota has been reported from single-species assays in laboratory studies than from whole ecosystem studies. The evidence is strong for effects of Pb on growth, reproduction, and survival in very diverse species, but considerable uncertainties exist in generalizing effects observed under particular, small-scale conditions, up to the ecosystem level of biological organization. At the ecosystem level, the presence of multiple stressors, variability in field conditions, and differences in bioavailability of Pb make it difficult to measure the magnitude of effects, and to quantify relationships between ambient concentrations of Pb and ecosystem response. However, the cumulative evidence that has been reported for Pb effects at higher levels of biological organization and for endpoints in single species with direct relevance to population and ecosystem level effects (i.e., development and reproduction, growth, survival) is sufficient to conclude that a causal relationship is likely to exist between Pb exposures and the alteration of species richness, species composition and biodiversity in terrestrial and freshwater ecosystems (Sections 6.3.12.7 and 6.4.12.7). Evidence is inadequate to conclude that there is a causal relationship between Pb exposure and effects at higher levels of biological organization in saltwater ecosystems (Section 6.4.21.7).

Ecosystem-level studies *in situ* are complicated by the frequent confounding of Pb exposure in Pb-polluted sites with other factors such as other trace metals and acidic deposition. In those natural systems, Pb is often found co-existing with other stressors, and observed effects may be due to cumulative toxicity. In laboratory studies and simulated ecosystems, where it is possible to isolate the effect of Pb, this metal has been shown to alter competitive behavior of species, predator-prey interactions, and contaminant avoidance. At higher levels of biological organization, these effects may change species abundance and community structure. Uptake of Pb into aquatic and terrestrial organisms and its effects on survival, growth, and reproductive endpoints at the organism level are expected to have ecosystem-level consequences. Where evidence of effects is observed at the ecosystem level of organization, evidence from lower levels brings consistency and plausibility for causality.

Most direct evidence of community and ecosystem level effects is from near stationary sources and contaminated sites where Pb concentrations are higher than typically observed in the environment. For terrestrial systems, evidence of impacts on natural ecosystems near smelters, mines, and other industrial sources of Pb has been assembled in previous decades. Those impacts include decreases in species diversity and changes in floral and faunal community composition. For freshwater systems, the literature focuses on evaluating ecological stress from Pb originating from urban and mining effluents

rather than atmospheric deposition. Some organisms exhibit contaminant avoidance behaviors when exposed to Pb-contaminated areas. For example, snails and fish avoid higher concentrations of Pb while frogs and toads lack avoidance response. Recent evidence, published since the 2006 Pb AQCD indicates that some species of worms will avoid Pb-contaminated soils (Langdon et al., 2005).These dynamics are likely to change species abundance and community structure at higher levels of biological organization.

Recent studies continue to demonstrate associations between Pb exposures and effects at higher levels of biological organization that were shown in field and microcosm studies in previous Pb AQCDs. Recent studies on plant and soil microbial communities and sediment-associated and aquatic plant communities increase the total number of types of ecological associations impacted by Pb. In terrestrial ecosystems, most studies show decreases in microorganism abundance, diversity, and function with increasing soil Pb concentration. Specifically, shifts in nematode communities, bacterial species, and fungal diversity have been observed. Furthermore, presence of arbuscular mycorrhizal fungi may protect plants growing in Pb-contaminated soils. Increased plant diversity ameliorated effects of Pb contamination on a microbial community.

In aquatic ecosystems, Pb effects reviewed in the 2006 Pb AQCD (U.S. EPA, 2006b) included reduced species abundance, richness and diversity, decreased primary productivity, and altered predator-prey interactions. Since the 2006 Pb AQCD, there is further evidence for effects of Pb in sediment-associated communities in both saltwater and freshwater systems. Community structure and nematode diversity were altered in a microcosm study with marine sediments (Mahmoudi et al., 2007). Sediment-bound Pb contamination appears to differentially affect members of the benthic invertebrate community, potentially altering ecosystem dynamics in small urban streams (Kominkova and Nabelkova, 2005). Although surface water Pb concentrations in monitored streams were determined to be very low, concentrations of the metal in sediment were high enough to pose a risk to the benthic community (e.g., 34-101 mg Pb/kg). These risks were observed to be linked to benthic invertebrate functional feeding group, with collector-gatherer species exhibiting larger body burdens of heavy metals than benthic predators and collector-filterers.

Changes to aquatic plant community composition have been observed in the presence of elevated surface water Pb concentrations. A shift toward more Pb-tolerant species is also observed in terrestrial plant communities near smelter sites (U.S. EPA, 2006b). Certain types of plants such as rooted and submerged aquatic plants may be more susceptible to aerially-deposited Pb resulting in shifts in Pb community composition. High Pb sediment concentrations are linked to shifts in amphipod communities inhabiting plant structures.

1.8 Integration of Health and Ecological Effects

The health and ecological effects considered for causal determination are summarized in Table 1-4. The health outcomes were those related to the nervous, cardiovascular, renal, and immune system, effects on heme synthesis and RBC function, reproductive and developmental effects, and cancer. The ecological endpoints considered for causal determination were: community and ecosystem level effects, reproductive and developmental effects, growth, survival, neurobehavioral effects, hematological effects, and physiological stress. The evidence relating to specific ecological endpoints is also integrated across aquatic and terrestrial habitats. Further, the substantial overlap between the ecological and health endpoints considered in the causal determinations allowed the integration of the evidence across these disciplines.

Table 1-4 Summary of causal determinations for health and ecological effects.

Outcome/Effect	Human Health Causal Determination[a]	Ecological Receptors Causal Determination[a]
Nervous System Effects[b]	Causal Relationship: Cognition, Attention, Impulsivity and Hyperactivity in Children	Likely Causal Relationship: Neurobehavioral Effects in Terrestrial and Freshwater Invertebrates and Vertebrates
Cardiovascular Effects	Causal Relationship: Hypertension and Coronary Heart Disease	N/A[e]
Renal Effects	Likely Causal Relationship: Reduced Kidney Function	N/A[e]
Immune Effects	Likely Causal Relationship: Atopic and Inflammatory Conditions, Decreased Host Resistance	N/A[e]
Hematological Effects[c]	Causal Relationship: RBC Function and Survival, Altered Heme Synthesis	Causal Relationship: ALAD Activity in Terrestrial and Freshwater Vertebrates Likely Causal Relationship: ALAD activity in Freshwater Invertebrates
Reproductive and Developmental Effects[d]	Causal Relationship: Development and Male Reproductive Function	Causal Relationship: Invertebrates and Vertebrates
Cancer	Likely to be a causal relationship	N/A[e]

Table 1-4 (Continued): Summary of causal determinations for health and ecological effects.

Outcome/Effect	Human Health Causal Determination[a]	Ecological Receptors Causal Determination[a]
Mortality	N/A[e] (The strongest evidence of Pb-induced mortality in humans was observed for cardiovascular disease related mortality and this evidence was considered in determining the causal relationship between Pb exposure and coronary heart disease.)	Causal Relationship: Survival Terrestrial Invertebrates and Freshwater Invertebrates and Vertebrates Likely Causal Relationship: Terrestrial Vertebrates
Growth	N/A[e] (There is mixed evidence from toxicological and epidemiologic studies of Pb effects on postnatal growth, which was considered in determining the causal association between Pb exposure and developmental effects.)	Causal Relationship: Terrestrial Plants and Freshwater Invertebrates Likely Causal Relationship: Freshwater Plants and Terrestrial Invertebrates
Physiological Stress	N/A[e] (In Human Health, oxidative stress was considered as an upstream event in the modes of action of Pb, leading downstream to various effects. Ecological literature commonly uses oxidative stress as a proxy indicator of overall fitness, and thus treats it as an effect.)	Causal Relationship: Terrestrial Plants Likely Causal Relationship: Terrestrial and Freshwater Invertebrates and Vertebrates and Freshwater Plants
Community and Ecosystem Level Effects	N/A[e]	Likely Causal Relationship: Terrestrial and Freshwater Ecosystems

[a] In drawing conclusions regarding the causal relationship between Pb exposure and human health effects, evidence in the range of relevant pollutant exposures or biomarker levels was considered. Evidence from toxicological studies of effects observed in experimental animals at biomarker levels (e.g. blood Pb) comparable to those currently experienced by the U.S. general population were emphasized. Generally, studies with dietary exposures resulting in blood Pb levels within one order of magnitude above the upper end of the distribution of U.S. blood Pb levels were considered in forming concusions, with the majority of studies reporting blood Pb levels below 30 µg/dLIn forming conclusions, Ecological effects observed at or near ambient Pb concentrations measured in soil, sediment and water in the most recent available studies (Table 1-1) were emphasized and studies generally within one to two orders of magnitude above the reported range of these values were considered in the body of evidence for terrestrial, freshwater and saltwater ecosystems.

[b] In ecological receptors, the causal determination was developed considering neurobehavioral effects that can be observed in toxicological studies of animal models and studies of ecological effects in vertebrates and invertebrates.

[c] The ecological evidence considered for the causal determination included ALAD activity, blood cell count, and altered serum profiles.

[d] For health effects the strongest evidence was for delayed onset of puberty and detrimental effects on sperm. In the ecological literature, a wide range of endpoints, including embryonic development, multigenerational studies, delayed metamorphosis, and altered steroid profiles, was considered.

[e] N/A, not applicable, i.e., Endpoints were not directly comparable for the health and ecological evidence.

1.8.1 Modes of Action Relevant to Downstream Health and Ecological Effects

The diverse health and ecological effects of Pb are mediated through multiple, interconnected modes of action. This section summarizes the principal cellular/subcellular effects contributing to modes of action for human health endpoints associated with Pb exposure and the concentrations at which those effects are observed. Then, effects of Pb observed in aquatic and terrestrial species (Section 1.7) are evaluated along with evidence from epidemiological and laboratory animal studies to determine the extent to which common modes of action can be inferred from the observed effects. The rationale for this approach is that the mechanism of Pb toxicity is likely conserved from invertebrates to vertebrates to humans in some organ systems.

Each of the modes of action discussed in Section 4.2 has the potential to contribute to the development of a number of Pb-induced health effects (Table 1-4). Evidence for the majority of these modes of action is observed at low blood Pb levels in humans and laboratory animals, between 2 and 5 µg/dL, and at doses as low as the picomolar range in animals and cells. The concentrations eliciting the modes of action (reported in Table 1-5) are drawn from the available data and do not imply that these modes of action are not acting at lower exposure levels or that these doses represent the threshold of the effect. Also, the data in presented this table does not inform regarding the exposure frequency and duration required to elicit a particular MOA.

Ecosystem studies have presented evidence for the occurrence of many of these modes of action in animals, and to some degree in plants, however the connection to ecological outcomes must usually be inferred because ecological studies are typically not designed to address mode of action directly. The level at which Pb elicits a specific effect is more difficult to establish in terrestrial and aquatic systems due to the influence of environmental variables on Pb bioavailability and toxicity and substantial species differences in Pb susceptibility.

Table 1-5 Modes of action, their related health effects, and information on concentrations eliciting the MOAs.

Mode of Action [Related Health Effects (ISA Section)]	Concentrations or Doses (Conditions)[a]	
	Blood Pb	**Dose**
Altered Ion Status [All Health Effects of Pb; Chapter 4]	3.5 µg/dL (Mean in cord blood; association with cord blood Ca^{2+}ATPase pump activity) Huel et al. (2008)	0.00005 µM free Pb^{2+} (In vitro; 30 minutes; calmodulin activation assay) Kern et al. (2000)
Protein Binding [Renal (4.5), Heme Synthesis and RBC Function (4.7)]	17.0 µg/dL (Concurrent mean in adult workers with wildtype metallothionein expression; increased BP susceptibility) Chen et al. (2010a)	50 µM Pb glutamate (In vitro; 24 hours; increased nuclear protein in neurological cell) Klann and Shelton (1989)
Oxidative Stress [All Heath Effects of Pb (Chapter 4)]	5.4 µg/dL (Concurrent mean in adult male workers; decreased CAT activity in blood) Conterato et al. (2013)	0.1 µM Pb acetate (In vitro; 48 hours; decreased cellular GSH in neuroblastoma cells) Chetty et al. (2005)
Inflammation [Nervous System (4.3), Cardiovascular (4.4), Renal (4.5), Immune (4.6), Respiratory (4.6.5 and 4.9.6), Hepatic (4.9.1)]	Among males with concurrent blood Pb ≥ 2.5 µg/dL (Increased serum TNF-α and blood WBC count) Kim et al. (2007)	0.01 µM Pb acetate (In vitro; 48 hours; increased cellular PGE_2 in neuroblastoma cells) Chetty et al. (2005)
Endocrine Disruption [Reproductive and Developmental Effects (4.8), Endocrine System (4.9.3) , Bone and Teeth (4.9.4)]	1.7 µg/dL (Lowest blood Pb level at which a relationship could be detected in adult women with both ovaries removed; increased serum follicle stimulating hormone [FSH]) Krieg (2007)	10 µM Pb nitrate (In vitro; 30 minutes; displaced GHRH binding to rat pituitary receptors) Lau et al. (1991)
Cell Death/Genotoxicity [Cancer (4.10), Reproductive and Developmental Effects (4.8), Bone and Teeth (4.9.4)]	3.3 µg/dL (Concurrent median in adult women; increased rate of hypoxanthine guanine phospho ribosyltransferase reporter gene [HPRT] mutation frequency) Van et al. (2004)	0.03 µM Pb acetate (In vitro; 18 hours; increased formation of micronuclei) Bonacker et al. (2005)

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

The alteration of cellular ion status (including disruption of Ca^{2+} homeostasis, altered ion transport mechanisms, and perturbed protein function through displacement of metal cofactors) appears to be the major unifying mode of action underlying all subsequent modes of action in plants, animals, and humans (Figure 4-1). Pb can interfere with endogenous cation homeostasis, necessary as a cell signal carrier mediating normal cellular functions. Pb is able to displace metal ions, such as Zn, Mg, and Ca^{2+}, from proteins due to the flexible coordination numbers and multiple ligand binding ability of Pb, leading to abnormal conformational changes to proteins and altered protein function. Disruption of ion transport leading to increased intracellular Ca^{2+} levels is due in part to the alteration of the activity of transport channels and proteins, such as Na^{+}/K^{+}ATPase and voltage-sensitive Ca^{2+} channels. Pb can interfere with these proteins through direct competition between Pb and the native metals present in the protein metal binding domain or through disruption of proteins important in Ca^{2+}-dependent cell signaling, such as protein kinase C (PKC) or calmodulin.

This competition between metals has been reported not only in human systems, but also in fish, snails, and plants. Altered Ca^{2+} channel activity and binding of Pb with Na^{+}/K^{+}ATPase in the gills of fish disrupts the Na^{+} and Cl^{-} homeostasis, which may lead to ionoregulatory failure and death. Ca^{2+} influx and ionoregulation has also been shown to be inhibited by Pb exposure in a sensitive species of snail, leading to a reduction in snail growth. In plants, substitution of the central atom of chlorophyll, Mg, by Pb prevents light-harvesting, resulting in a breakdown of photosynthesis. Pb-exposed animals also have decreased cellular energy production due to perturbation of mitochondrial function.

Disruption of ion transport not only leads to altered Ca^{2+} homeostasis, but can also result in perturbed neurotransmitter function. Evidence for these effects in Pb-exposed experimental animals and cell cultures has been linked to altered neurobehavioral endpoints and other neurotoxicity. Neurobehavioral changes that may decrease the overall fitness of the organism have also been observed in aquatic and terrestrial invertebrate and vertebrate studies. There is evidence in tadpoles and fish to suggest Pb may alter neurotransmitter concentrations, possibly resulting in some of these neurobehavioral changes.

Altered cellular ion status following Pb exposure can result in the inhibition of heme synthesis. Pb exposure is commonly associated with altered hematological responses in aquatic and terrestrial invertebrates, experimental animals, and human subjects. The proteins involved in heme synthesis that are affected by Pb are highly conserved across species, which may account for the common response seen in human health and ecological studies. This evolutionarily conserved response to Pb is likely the result of the

competition of Pb with the necessary metal cofactors in the proteins involved in heme synthesis.

Although Pb will bind to proteins within cells through interactions with side group moieties, thus potentially disrupting cellular function, protein binding of Pb may represent a mechanism by which cells protect themselves against the toxic effects of Pb. Intranuclear and intracytosolic inclusion body formation has been observed in the kidney, liver, lung, and brain following Pb exposure in experimental animals. A number of unique Pb binding proteins have been detected, constituting the observed inclusion bodies. The major Pb binding protein in blood is ALAD with carriers of the ALAD-2 allele potentially exhibiting higher Pb binding affinity. Inhibition of ALAD activity is a widely recognized response to Pb in environments where Pb is present and is considered to be biomarker of Pb exposure in both terrestrial and aquatic biota. Additionally, metallothionein is an important protein in the formation of inclusion bodies and mitigation of the toxic effects of Pb. Protein binding of Pb is a recognized mechanism of Pb detoxification in some terrestrial and aquatic biota. For example, plants can sequester Pb through binding with phytochelatin and some fish have the ability to store accumulated Pb in heat-stable proteins.

A second major mode of action of Pb is the development of oxidative stress, due in many instances to the antagonism of normal metal ion functions. Disturbances of the normal redox state of tissues can cause toxic effects and is involved in the majority of health and ecological outcomes observed after Pb exposure. The origin of oxidative stress produced after Pb exposure is likely a multi-pathway process. Studies in humans and experimental animals provide evidence to conclude that oxidative stress results from oxidation of δ-ALA, NAD(P)H oxidase activation, membrane and lipid peroxidation, and antioxidant enzyme depletion. Evidence of increased lipid peroxidation associated with Pb exposure exists for many species of plants, invertebrates, and vertebrates. Enhanced lipid peroxidation can also result from Pb potentiation of Fe^{2+} initiated lipid peroxidation and alteration of membrane composition after Pb exposure. Increased Pb-induced ROS will also sequester and inactivate biologically active nitric oxide or nitric oxide radical (NO), leading to the increased production of the toxic product nitrotyrosine, increased compensatory NOS, and decreased sGC protein. Pb-induced oxidative stress not only results from increased ROS production but also through the alteration and reduction in activity of the antioxidant defense enzymes. The biological actions of a number of these enzymes are antagonized due to the displacement of the protein functional metal ions by Pb. Increased ROS are often followed by a compensatory and protective upregulation in antioxidant enzymes, such that this observation is indicative of oxidative stress conditions. A number of studies in plants, invertebrates, and vertebrates present evidence of increased antioxidant enzymes with Pb exposure. Additionally, continuous ROS

production may overwhelm this defensive process leading to decreased antioxidant activity and further oxidative stress and injury.

In a number of organ systems Pb-induced oxidative stress is accompanied by misregulated inflammation. Pb exposure will modulate inflammatory cell function, production of proinflammatory cytokines and metabolites, inflammatory chemical messengers, and proinflammatory signaling cascades. Cytokine production is skewed toward the production of proinflammatory cytokines like TNF-α and IL-6 as well as leading to the promotion of Th2 response and suppression of Th1cytokines and Th1-related responses.

Pb is a potent endocrine disrupting chemical. Steroid receptors and some endocrine signaling pathways are known to be highly conserved over a broad expanse of animal phylogeny. Pb can disrupt the HPG axis evidenced in humans, other mammals, and fish, by a decrease in serum hormone levels, such as FSH, LH, testosterone, and estradiol. Pb interacts with the hypothalamic-pituitary level hormone control causing a decrease in pituitary hormones, altered growth dynamics, inhibition of LH secretion, and reduction in StAR protein. Pb has also been shown to alter hormone receptor binding likely due to interference of metal cations in secondary messenger systems and receptor ligand binding and through generation of ROS. Pb disrupts hormonal homeostasis in invertebrates necessary for reproduction and development. Pb also may disrupt the HPT axis by alteration of a number of thyroid hormones, possibly due to oxidative stress. These studies have been conducted in humans and other animals, including cattle; however the results of these studies are mixed.

Genotoxicity and cell death has been investigated after Pb exposure in humans, animals, plants, and cell models. High level Pb exposure to humans leads to increased DNA damage, however lower blood Pb levels have been associated with these effects in experimental animals and cells. Reports vary on the effect of Pb on DNA repair activity; however, a number of studies report decreased repair processes following Pb exposure. There is some evidence in plants, earthworms, freshwater mussels and fish for DNA damage associated with Pb exposure. There is evidence of mutagenesis and clastogenicity in highly exposed humans, however weak evidence has been shown in animals and cells based systems. Human occupational studies provide limited evidence for micronucleus formation (blood Pb levels >10 µg/dL), supported by Pb-induced effects in both animal and cell studies. Micronucleus formation has also been reported in amphibians. Animal and plant studies have also provided evidence for Pb-induced chromosomal aberrations. The observed increases in clastogenicity may be the result of increased oxidative damage to DNA due to Pb exposure, as co-exposures with antioxidants ameliorate the observed toxicities. Limited evidence of epigenetic effects is available, including DNA

methylation, mitogenesis, and gene expression. Altered gene expression may come about through Pb displacing Zn from multiple transcriptional factors, and thus perturbing their normal cellular activities. Consistently positive results have provided evidence of increased apoptosis of various cell types following Pb exposure.

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

1.9 Policy Relevant Considerations

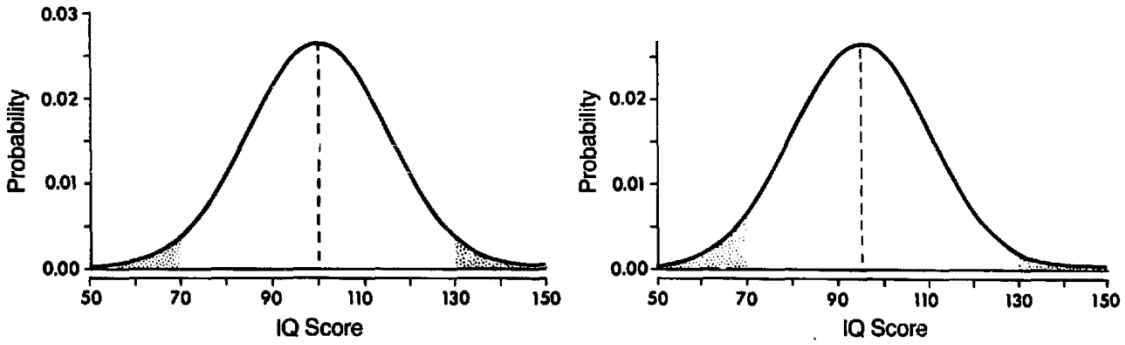
1.9.1 Public Health Significance

The rationale for establishing the public health significance of the various health endpoints associated with Pb exposure is multifaceted. The 2006 Pb AQCD (U.S. EPA, 2006b) concluded that neurodevelopmental effects in children and cardiovascular effects in adults were among the effects best substantiated as occurring at the lowest blood Pb levels, and that these categories of effects were clearly of the greatest public health concern. The evidence reviewed in the current assessment supports and builds upon this conclusion. Evidence in a few cohorts of children that indicated the supralinear concentration-response blood Pb-FSIQ relationships, did not identify a threshold for Pb-associated neurodevelopmental effects in the range of blood Pb levels examined (Sections 1.9.3 and 4.3.13).

The World Health Organization (WHO) definition of "health" is "the state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity" (WHO, 1948). By this definition, decrements in health status that are not severe enough to result in the assignment of a clinical diagnosis might reflect a decrement in the well-being of an individual. Further, deficits in subtle indices of health or well-being may not be observable except in aggregate, at the population level, so the critical distinction between population and individual risk is essential for interpreting the public health significance of study findings. This concept of population risk is relevant to the interpretation of findings regarding both IQ and blood pressure in the assessment of their public health significance.

Weiss et al. (1988) discusses the hypothetical impact of a small shift in a population distribution of IQ Score. As shown in Figure 1-1, these authors anticipate that even a small shift in the population mean IQ may be significant from a public health perspective because such a shift could yield a larger proportion of individuals functioning in the low

range of the IQ distribution, which is associated with increased risk of educational, vocational, and social failure (Section 4.3.13), as well as reduce the proportion of individuals with high IQ scores.



Note: Two distributions of intelligence test scores. (Left): Based on a mean of 100 (the standardized average, with a standard deviation of 15. (Right):Demonstrating a 5% reduction model, based on a mean score of 95. This is a conceptual model that assumes that the incremental concentration-response between Pb exposure and IQ is similar across the full range of IQ, and is not based on actual data. The figure shows that the effect of a small shift in population mean IQ score may result in a larger proportion of individuals with IQ scores below 70 and a smaller proportion with IQ scores above 130.

Source: Reprinted with permission of Elsevier; from Weiss et al. (1988)

Figure 1-1 Distributions of IQ scores.

It is also important to note that the change in a population mean observed in an epidemiologic study may be small compared to the standard error of measurement for the outcome. Measurement error in the outcome can affect the likelihood of detecting an association but if a study is large enough it will have adequate statistical power to detect small changes at the population level. Bias may be introduced if the measurement error of the outcome is highly correlated with the exposure. There is no evidence to suggest that individuals with higher blood Pb levels test systematically lower than their true IQ.

Pb-associated changes in blood pressure also increase an individual's risk for health effects that are of greater clinical consequence than is suggested by a small individual change in blood pressure. Nawrot et al. (2002) found that a doubling of blood Pb was associated with an approximate 1 mmHg increase in systolic blood pressure. Results from the Framingham Heart Study show that higher levels of blood pressure, even within the nonhypertensive range, increase the risk of cardiovascular disease (Kannel, 2000a, b). A continuous graded increase in cardiovascular risk is observed as blood pressure increases, with no evidence of a threshold value. Most events arise not in the most severe

cases, but mainly in those with high normal blood pressure (i.e., mild hypertension). Kannel (2000a) emphasized that systolic blood pressure exerts a strong influence on more serious cardiovascular events, as it is the primary cause of hypertension and its adverse cardiovascular sequelae. Pb-associated effects on cardiovascular morbidity outcomes such as ischemic heart disease (Section 4.4.3.6) and mortality (Section 4.4.5) have also been observed. In addition, some groups within the population can be at greater risks for cardiovascular effects; as summarized in Chapter 5, there is evidence for increased cardiovascular effects based on race/ethnicity and several genetic markers. Overall, while some of the specific health endpoints that have been associated with Pb exposure are small physiological changes in an individual, these changes can represent substantial risk at the population level.

1.9.2 Air-Pb–to–Blood-Pb Relationships

The 1986 Pb AQCD described epidemiologic studies of relationships between air Pb and blood Pb. Much of the pertinent earlier literature for children described in the 1986 Pb AQCD was included in a meta-analysis by Brunekreef (1984). Based on the studies available at that time, the 1986 Pb AQCD concluded that "the blood Pb versus air Pb slope β is much smaller at high blood and air levels." This is to say that the slope β was much smaller for occupational exposures where high blood Pb levels (>40 μg/dL) and high air Pb levels (much greater than 10 $\mu g/m^3$) prevailed relative to lower environmental exposures which showed lower blood Pb and air Pb concentrations (<30 μg/dL and <3 $\mu g/m^3$). For those environmental exposures, it was concluded that the relationship between blood Pb and air Pb "…for direct inhalation appears to be approximately linear in the range of normal ambient exposures (0.1-2.0 $\mu g/m^3$)" (pp 1–98 of the 1986 Pb AQCD). In addition to the meta-analysis of Brunekreef (1984), more recent studies have provided data from which estimates of the blood Pb-air Pb slope can be derived for children (Table 1-6, Table 3-12). The range of estimates from these studies is 4-9 μg/dL per $\mu g/m^3$, which encompasses the estimate from the Brunekreef (1984) meta-analysis. Most studies have described the blood Pb-air Pb relationship as either log-log (Schnaas et al., 2004; Hayes et al., 1994; Brunekreef, 1984), which predicts an increase in the blood Pb-air Pb slope with decreasing air Pb concentration or linear (Hilts, 2003; Tripathi et al., 2001; Schwartz and Pitcher, 1989), which predicts a constant blood Pb-air Pb slope regardless of air Pb concentrations. These differences may simply reflect model selection by the investigators; alternative models are not reported in these studies.

The blood Pb-air Pb slope may also be affected in some studies by the inclusion of parameters (e.g., soil Pb) that may account for some of the variance in blood Pb attributable to air Pb. Other factors that likely contribute to the derived blood Pb-air Pb

slope include differences in the populations examined and Pb sources, which varied among individual studies. See Section 3.5 for a detailed discussion of studies that inform air Pb-to blood-Pb relationships.

Table 1-6 Summary of estimated slopes for blood Pb to air Pb relationships in children.

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

Table 1-6 (Continued): Summary of estimated slopes for blood Pb to air Pb relationships in children.

Reference	Study Methods	Model Description	Blood Pb–Air Pb Slope[a]
Tripathi et al. (2001)	**Location:** Mumbai, India (multiple residential locations) **Years:** 1984-1996 **Subjects:** 6-10 yr (N = 544) **Analysis:** Regression of residential location-specific average blood Pb and air Pb data	**Model:** Linear **Blood Pb:** 8.6-14.4 µg/dL (GM range for residential locations) **Air Pb:** 0.11-1.18 µg/m^3 (GM range for residential locations)	3.6 (0.45)[i]

[a]Slope is predicted change in blood Pb (µg/dL per µg/m^3) evaluated at ± 0.01 µg/m^3 from central estimate of air Pb for the study (shown in parentheses). The central estimate for the Brunekreef (1984) study, is the median of air Pb concentrations, since it was a meta-analysis; for all other studies the mean is presented. For multiple regression models, this is derived based only on air Pb coefficient and intercept. Depending on the extent to which other variables modeled also represent air Pb, this method may underestimate the slope attributable to air pathways. In single regression models, the extent to which non-modeled factors, unrelated to air Pb exposures, exert an impact on blood Pb that co-varies with air Pb may lead to the slope presented here to over represent the role of air Pb.

[b]ln(PbB) = ln(PbA) × 0.3485 + 2.853

[c]ln(PbB) = ln(PbA) × 0.2159 + 2.620

[d]ln(PbB) = ln(PbA) × 0.24 + 3.17

[e]PbB = PbA × 7.0

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

[g]PbB = PbA × 8.6

[h]Based on data for the U.S. [1986 Pb AQCD, (U.S. EPA, 1986a)].

[i]PbB =Pb A × 3.6

GM, geometric mean; GSD, geometric standard deviation; PbB, blood Pb concentration (µg/dL); PbA, air-Pb concentration (µg/m^3)

1.9.3 Concentration-Response Relationships for Human Health Effects

Concentration response (C-R) relationships have been examined most extensively in studies of neurodevelopmental effects in children. Although relatively few studies examined the shape of the concentration-response relationship between Pb in blood or bone and effects in adults, several recent studies of adult endpoints (i.e., cognitive function, cardiovascular and mortality effects) add to the evidence. Some of the populations examined (e.g., NHANES, NAS) are likely to have had higher past than recent Pb exposure. Other populations (e.g., worker populations) studied have ongoing exposure to Pb. As described elsewhere in the document (Sections 3.3, 4.3, 4.4, and 4.5), the interpretation of the study findings depends on the exposure history and the choice of the biomarker in the context of what is known about that exposure history. There is uncertainty regarding the frequency, duration, timing and level of exposure contributing to the blood Pb or bone Pb levels in the adult populations studied.

Cognitive and Behavioral Effects in Children

With each successive Pb AQCD and supplement, the epidemiologic and toxicological study findings show that progressively lower blood Pb levels or Pb exposures are associated with cognitive deficits in children (Section 4.3.13). For example, effects were observed in association with blood Pb levels in the range of 10-15 µg/dL in the 1986 Addendum (U.S. EPA, 1986c) and 1990 Supplement (U.S. EPA, 1990a), and 10 µg/dL and lower in the 2006 Pb AQCD (U.S. EPA, 2006b). No evidence of a threshold for the effects of Pb on neurodevelopmental effects has been reported across the range of blood Pb levels examined in epidemiologic studies.

Compelling evidence for a larger decrement in cognitive function per unit increase in blood Pb among children with lower mean blood Pb concentrations compared to children with higher mean blood Pb concentrations was presented in the 2006 Pb AQCD. Key evidence was provided by studies that examined prenatal or early childhood blood Pb levels or considered peak blood Pb levels in schoolaged children or concurrent blood Pb levels in young children age 2 years (Section 4.3.12, Figure 4-16, and Table 4-16) (Téllez-Rojo et al., 2006) as well as the international pooled analysis of seven prospective cohort studies by Lanphear et al. (2005), a subsequent reanalysis of these data focusing on the shape of the concentration response function (Rothenberg and Rothenberg, 2005).

Attenuation of C-R relationships at higher exposure or dose levels has been reported in the occupational literature for a range of exposures. Reasons proposed to explain the attenuation include greater exposure measurement error and saturation of biological mechanisms at higher levels as well depletion of the pool of susceptible individuals at higher exposure levels (Stayner et al., 2003). Possible explanations specific to nonlinear relationships observed in studies of Pb exposure in children include a lower incremental effect of Pb due to covarying risk factors such as low SES, poor caregiving environment, and higher exposure to other environmental factors (Schwartz, 1994), differential activity of mechanisms at different exposure levels, and confounding by omitted or misspecified variables. Review of the evidence did not reveal a consistent set of covarying risk factors to explain the differences in blood Pb IQ C-R relationship across high and low Pb exposure groups observed in epidemiologic studies. Nonlinear concentration-response relationships including U- or inverted U-shaped curves for various endpoints, including those related to cognitive impairment were demonstrated in the toxicological literature. However, these toxicological findings are distinct from epidemiologic findings of supralinear relationships in that some U- or inverted U-shaped relationships do not indicate Pb-induced impairments at higher exposure concentrations.

The supralinear relationship reported in multiple prospective studies does not provide evidence of a threshold for Pb-associated cognitive function decrements. For example, as

detailed in Section 4.3.12, higher blood Pb levels at age 2 years were associated with FSIQ decrements in children aged 10 years whose blood Pb levels were in the range of 1.0-9.3 µg/dL, e.g.(Bellinger, 2008). Supporting evidence was provided by Pb-associated decrements in academic performance observed in fourth grade children with earlier childhood blood Pb levels categorized as 2 µg/dL versus 1 µg/dL (Miranda et al., 2009; 2007a). The lack of a reference population with blood Pb levels reflecting still lower Pb exposures limits the ability to identify a threshold in the current population. Toxicological studies showed that lower Pb exposures (e.g., 50 ppm in drinking water) induced learning and memory impairments in animals compared to control exposures or higher Pb exposures (e.g., 150 ppm). Additional toxicological evidence suggests that mechanisms may be differentially activated at lower and higher Pb exposures, and reduced long-term potentiation (LTP) and hippocampal glutamate release with lower Pb exposures may provide explanation for the impaired learning and memory observed with lower Pb exposures in some animal studies.

Studies of Pb Effects in Adults

The shape of the C-R function (e.g., linear versus non-linear) was not examined in most studies of the association of Pb biomarkers with cognitive function in adults (Sections 4.3.2.7 and 4.3.13). Log-linear models were used to fit the data in NHANES analyses. Nonlinearity in the relationship between bone Pb and cognitive function among participants in the BMS and NAS cohorts was examined with the use of quadratic terms, penalized splines, or visual inspection of bivariate plots. There was some evidence for nonlinearity in prospective analyses of the NAS cohort (Figure 4-7 and Figure 4-8), but not all results indicated a larger decrement in cognitive function per unit increase in bone Pb level in the lower end of the bone Pb distribution. In the BMS cohort, observation of a statistically nonsignificant quadratic bone Pb term indicated that a linear model fit the relationship between tibia Pb level and various tests of cognitive function.

A meta-analysis of human studies found that each doubling of blood Pb level (between 1 and >40 µg/dL measured concurrently in most studies of adults for which past exposures were likely higher than current exposures) was associated with a 1 mmHg increase in systolic BP and a 0.6 mmHg increase in diastolic BP (Nawrot et al., 2002). In this analysis, effect sizes were adjusted for the purpose of pooling them depending on whether a linear or log (common or natural) linear model was used. The functional form of the C-R relationship was examined in few individual studies of cardiovascular effects (Section 4.4.2.1). Weaver (2010), reported that a logarithmic function of blood Pb level better described data from a cohort of Korean workers than the linear form. Only a small number of studies that focused on Pb-induced hypertension in experimental animals have

included more than two exposure concentrations, and these studies appear to show a nonlinear concentration-response (Figure 4-21).

Studies investigating both all-cause and cardiovascular mortality report both linear and non-linear relationships (Section 4.4.5). Although associations are consistently reported, findings regarding the shape of the C-R relationship between blood Pb level and mortality in NHANES analyses were mixed. For example, in the NAS cohort, C-R relationships between bone Pb and mortality were approximately linear for patella Pb on the log(heart rate [HR]) scale for all cardiovascular disease (CVD), but appear nonlinear for IHD (Weisskopf et al., 2009). It is important to note the wide confidence limits, which increase uncertainty at the lower and upper bounds of patella Pb levels. The strongest associations were observed between mortality and baseline patella Pb concentration while tibia Pb levels were more weakly associated with CVD mortality.

1.9.4 Pb Exposure and Neurodevelopmental Deficits in Children

As discussed in Section 3.3.5, blood Pb may reflect both recent exposures as well as past exposures because Pb is both taken up by and released from the bone. The relative proportion of blood Pb from recent versus past exposure is uncertain in the absence of specific information about the pattern of exposure contributing to observed blood Pb levels. This uncertainty is greater in adults and older children, than in young children who do not have lengthy exposure histories. Several lines of evidence, which are summarized below, inform the interpretation of epidemiologic studies of young children with regard to the patterns of exposure that contribute to observed health effects.

Pb can cross the placenta to affect the developing fetal nervous system and fetal Pb exposure can occur from recent maternal exposure or from mobilization of bone Pb stores from past exposures (Section 3.2.2.4). In very young children, ages <2 years, decrements in mental development, as assessed with MDI, was associated with higher prenatal (maternal and cord) and concurrent blood Pb levels (Section 4.3.2.2). Thus, both postnatal child and maternal Pb exposures may contribute to neurodevelopmental effects in children from infancy to age 2 years. There is some evidence that the relative influence of maternal Pb levels on postnatal blood Pb level is substantially reduced soon after birth (Section 3.4). There was also a good correlation between child blood Pb level and child hand Pb loading ($R^2 = 0.70$) in a study following children living in a contaminated area, indicating the influence of concurrent Pb exposures on blood Pb during the early childhood years (Simon et al., 2007). In another study (Carbone et al., 1998) blood Pb levels of infants aged 6-12 months were significantly lower than their neonatal cord blood Pb levels (2.24 µg/dL versus 4.87 µg/dL). Among infants born with blood Pb levels

of greater than 7 μg/dL, who were followed for a week, there was a dramatic drop in the blood Pb (Carbone et al., 1998).

Epidemiologic studies consistently show that blood Pb levels measured during various lifestages or time periods throughout childhood, as well as averaged over multiple years during childhood, are associated with cognitive function decrements (Section 4.3.11). An international pooled analysis of seven prospective studies found that increments in concurrent and peak blood Pb levels were associated with a decrease in FSIQ measured between ages 5 and 10 years (Lanphear et al., 2005). In individual studies, postnatal (early childhood and concurrent) blood Pb levels are also consistently associated with cognitive function decrements in children and adolescents (Figure 4-2, Table 4-3, Table 4-14).

Exposure metrics based on blood Pb measurements at different ages in childhood are typically highly correlated. For example, analyses of serial blood Pb concentrations measured in longitudinal epidemiologic studies find relatively strong correlations (e.g., r = 0.5-0.8) among each child's individual blood Pb concentrations measured after 6-12 months of age (Section 3.3.2). Consequently, the relative importance of various exposure metrics, which are measured during different lifestages and time periods, is difficult to discern in epidemiologic studies. Evidence in rodents and monkeys, however, indicates that Pb exposures during multiple lifestages and time periods, including prenatal only, prenatal plus lactational, postnatal only, lifetime are observed to induce impairments in learning (Rice, 1992b, 1990; Rice and Gilbert, 1990b). These findings are consistent with the understanding that the nervous system continues to develop (i.e., synaptogenesis and synaptic pruning remains active) throughout childhood and into adolescence.

1.9.5 Reversibility and Persistence of Neurotoxic Effects of Pb

The 2006 Pb AQCD concluded that the human and animal evidence suggest that the neurotoxic effects of Pb are not generally reversible (U.S. EPA, 2006b). Chelation studies in humans and animals show that chelation decreases total body Pb burden, but does not necessarily exert evident effects on Pb-induced cognitive deficits. For example, analysis of multi-center study data indicates that medical interventions involving chelation therapy (e.g., Succimer use) do not fully reverse cognitive deficits associated with early Pb exposure (Liu et al., 2002).

The persistence of neurodevelopmental effects from comparatively low-level Pb exposure was considered in the 2006 Pb AQCD (U.S. EPA, 2006b), with some evidence suggesting that the effects of Pb on neurodevelopmental outcomes persisted into adolescence and young adulthood. The toxicological evidence continues to support a

range of effects with prenatal and early postnatal Pb exposures that persist to adulthood (Sections 4.3.2.3 and 4.3.3.1). In rats, persistent neurobehavioral deficits were observed with prenatal, preweaning, and postweaning Pb exposure. In monkeys, impairments were found in learning and short-term memory at ages 7 to 8 years (Rice and Karpinski, 1988) and in attention at ages 9 to 10 years (Gilbert and Rice, 1987) with lifetime Pb exposure that did not begin until postnatal day 400 and that produced peak blood-Pb levels <15 or 25 μg/dL and steady-state levels 11 and 13 μg/dL, indicating that postnatal juvenile Pb exposures were sufficient to produce neurodevelopmental deficits.

A number of mechanisms, including changes in neurogenesis, synaptogenesis and synaptic pruning, long term potentiation, and neurotransmitter function have been identified that provide biological plausibility for epidemiologic and toxicological findings of persistent cognitive and behavioral effects that result from Pb exposures during prenatal and early childhood periods. Furthermore, the normal dynamic and rapid rate of development that occurs early in life in the CNS makes insults early in life especially problematic in that they can permanently change the trajectory of brain development such that there are little or no compensatory pathways to replace the lost potential for proper brain development (Bayer, 1989).

The persistence of effects appears to depend on the duration and window of exposure as well as other factors that may affect an individual's ability to recover from an insult. Several studies have observed improved cognition in children with declining blood Pb levels (Hornung et al., 2009; Chen et al., 2005a; Liu et al., 2002; Ruff et al., 1993). There is evidence that some cognitive effects of prenatal Pb exposure may be transient and that recovery is greater among children reared in households with more optimal caregiving characteristics and in children whose concurrent blood Pb levels were low (Bellinger et al., 1990); the animal toxicology literature supports these findings using studies of Pb-exposed animals that live in enriched environments. However, the extent to which such improvement represents biological reversibility of Pb-related effects, the influence of enrichment related intervention, or the development of compensatory mechanisms remains uncertain.

Toxicological studies in the 2006 Pb AQCD highlighted the importance of Pb exposure during early life in promoting Alzheimer's-like pathologies in the adult rodent brain, with Pb-induced neurodegeneration and formation of neurofibrillary tangles in aged animals in which blood Pb levels had returned to control levels after an earlier life Pb exposure (U.S. EPA, 2006b). Sensitive windows of early life Pb exposure or a Pb biomarker and have been associated with changes in adulthood as demonstrated with animal models of neurodegeneration, i.e., neurofibrillary tangle formation. Behavioral or cognitive testing in these animal models has not been performed to assess these changes. These effects are

not reflective of concurrent blood Pb levels at the age of manifestation of the pathology but instead are associated with an earlier life Pb exposure.

1.9.6 Populations Potentially At-Risk for Health Effects

The NAAQS are intended to protect public health with an adequate margin of safety. In so doing, protection is provided for both the population as a whole and those groups potentially at increased risk for health effects from exposure to the air pollutant for which each NAAQS is set (Preface to this ISA). To facilitate the identification of populations at increased risk for Pb-related health effects, studies have evaluated various factors that may contribute to susceptibility and/or vulnerability to Pb. These factors include genetic background, race and ethnicity, sex, age, diet, pre-existing disease, SES, and characteristics that may modify exposure or the response to Pb. Table 1-7 and Table 5-5 provide an overview of the factors examined as potentially increasing the risk of Pb-related health effects based on the recent evidence integrated across disciplines. They are classified according to the criteria discussed in the introduction to Chapter 5.

In consideration of the evidence base as a whole (e.g., stratified and longitudinal analyses) and integrating across disciplines of toxicokinetics, exposure, and health, there is adequate evidence to conclude that children are an at-risk population. It is recognized that Pb can cross the placenta and affect the developing nervous system of the fetus (Section 3.2.2.4). Children may have increased exposure to Pb compared with adults because children's behaviors and activities (including increased hand-to-mouth contact, crawling, and poor hand-washing), differences in diets, and biokinetic factors. There is evidence of increased risk to the cognitive effects of Pb exposure during several lifestages and time periods throughout gestation, childhood, and into adolescence (Section 4.3.12). Findings from magnetic resonance imaging (MRI) studies indicate that normal brain development remains dynamic throughout adolescence, and epidemiologic studies have linked concurrent blood Pb level (as well as other blood Pb metrics) in adolescents to cognitive function decrements and delinquent or criminal behavior (Section 4.3.4). Delays in puberty onset (Section 4.8.1), and renal effects (Section 4.5.2.2), are also observed in association with concurrent blood Pb level in cross-sectional studies of adolescents. Since the populations of older children in these studies generally had higher past exposures, the current evidence does not clearly establish the link between a time and duration of Pb exposure and the observed health effects in the adolescent populations studied. Elevated biomarkers levels, which may be related to remobilization of stored Pb during bone loss and/or higher historical Pb exposures, are observed in older adults. Studies of older adults report inconsistent findings for effect measure modification of Pb-related mortality by age and no

modification of other health effects was studied. However, toxicological studies support the possibility of age-related differences in Pb-related health effects. The overall evidence, based on limited epidemiologic evidence but support from toxicological studies and differential exposure studies, is suggestive that older adults are potentially at risk of Pb effects. However, there are uncertainties related to the exposure profile associated with the effects in older populations.

Table 1-7 Summary of evidence for factors that potentially increase the risk of Pb-related health effects.

Factor Evaluated	Classification
Childhood (Sections 5.2.1, 5.3.1)	Adequate
Older Adulthood (Sections 5.2.1 and 5.3.1)	Suggestive
Sex (Sections 5.2.2, 5.3.2)	Suggestive
Genetics (Sections 5.3.3)	Suggestive
Pre-existing Disease[a] (Section 5.3.4)	Suggestive
Smoking Status (Section 5.3.5)	Inadequate
Socioeconomic Status (SES) (Sections 5.2.4, 5.3.6)	Suggestive
Race/Ethnicity (Sections 5.2.3, 5.3.7)	Adequate
Proximity to Pb Sources (Section 5.2.5)	Adequate
Residential Factors (Section 5.2.6)	Adequate
Body Mass Index (BMI) (Section 5.3.8)	Inadequate
Alcohol Consumption (Section 5.3.9)	Inadequate
Nutrition (Section 5.3.10)	Adequate
Stress (Section 5.3.11)	Suggestive
Maternal Self-Esteem (Section 5.3.12)	Inadequate
Cognitive Reserve[a] (Section 5.3.13)	Inadequate
Other Metals (Section 5.3.14)	Suggestive

[a]Possible mediator

The evidence regarding the other at-risk factors listed in the table above is summarized in detail in Section 5.4. Some studies suggest that males at some ages have higher blood Pb levels than comparably aged females; this was supported by stratifying the total sample of NHANES subjects. Sex-based differences appeared to be prominent among the adolescent and adult age groups but were not observed among the youngest age groups (1-5 years and 6-11 years). Studies of effect measure modification of Pb and various health endpoints by sex were inconsistent; although it appears that there are some

differences in associations for males and females. This is also observed in toxicological studies. Overall, there is suggestive evidence to conclude that sex is a potential at-risk factor, with adolescent and adult males typically demonstrating higher blood Pb levels, although evidence regarding health outcomes is limited due to inconsistencies in whether males or females are at greater risk of certain outcomes in relation to Pb exposure.

Regarding race and ethnicity, recent data suggest that the difference in blood Pb levels between black and white subjects is decreasing over time, but black subjects still tend to have higher Pb body burden and Pb exposures than white subjects. Compared to whites, non-white populations were observed to be more at risk of Pb-related health effects. Studies of race/ethnicity provide adequate evidence that race/ethnicity is an at-risk factor based on the higher exposure observed among non-white populations and some modification observed in studies of associations between Pb levels and health effects. For example two investigators report (Table 5-5), reported modification by race/ethnicity in an analysis of hypertension among NHANES III participants. Muntner et al. (2005) sons of the highest quartile of blood Pb to the lowest, the odds ratio for hypertension was 1.54 (95% CI: 0.99, 2.39) among Mexican Americans, 1.44 (95% CI: 0.89, 2.32) among Non-Hispanic Blacks and 1.10 (95% 0.87, 1.41) among Non-Hispanic Whites (Scinicariello et al., 2010).

The gap between SES groups with respect to Pb body burden appears to be diminishing. However, biomarkers of Pb exposure have been shown to be higher among lower SES groups even in recent studies in which differences among SES groups have lessened. Studies of SES and its relationship with Pb-related health effects are few and report inconsistent finding regarding low SES as a potential at-risk factor. Overall, the evidence is suggestive that low SES is a potential at-risk factor for Pb-related health effects.

There is adequate evidence that proximity to areas with Pb sources, including areas with large industrial sources, is associated with increased Pb exposure. Relatively high concentrations of ambient air Pb have been measured near sources, compared with large urban areas without sources and high Pb exposures have been documented near Superfund sites. NHANES analyses report increased Pb biomarker levels related to increase house dust Pb levels, homes built after 1950, and renovation of pre-1978 homes. Thus, there is adequate evidence that residing in a residence with sources of Pb exposures will increase the risk of Pb exposure and associated health effects.

There is suggestive evidence to conclude that various genetic variants potentially modify the associations between Pb and health effects. Epidemiologic and toxicological studies reported that ALAD variants may increase the risk of Pb-related health effects. Other genes examined whose variants may also affect risk of Pb-related health effects were VDR, DRD4, GSTM1, TNF-α, eNOS, and HFE. Overall the interaction between genetic

variants and Pb exposure were examined in a small number of studies and these types of analysis are potentially vulnerable to type II error if multiple statistical tests are conducted. However, there may be a large potential impact of Pb exposure in specific at-risk populations carrying specific gene variants. For example, Scinicariello et al. (2010) found that Non-Hispanic white carriers of the ALAD2 genetic variant in the highest blood Pb quartile had a 2-fold higher risk of hypertension compared with ALAD1 homozygous individuals (OR:2.00 [95%CI: 1.12, 3.55]). No evidence of effect modification of the association of Pb with blood pressure by ALAD genotype was observed in an occupational study of Korean Pb workers, however (Weaver et al., 2008). NAS subjects with the H63D polymorphism of the HFE gene had a larger Pb-associated increase in pulse pressure compared to those with the C282Y variant [i.e., 3.3 mmHg increase (95%CI: 0.16, 6.46) versus an 0.89 mmHg increase (95%CI 0-5.24) per 13 μg/g increase in tibia Pb (Zhang et al., 2010a)].

Diets sufficient in minerals such as calcium (Ca^{2+}), iron (Fe), and zinc (Zn) offer some protection from Pb exposure by preventing or competing with Pb for absorption in the GI tract. Additionally, those with Fe deficiencies were observed to be an at-risk population for Pb-related health effects in both epidemiologic and toxicological studies. Thus, there is adequate evidence across disciplines that some nutritional factors contribute to a population being at increased risk. Other nutritional factors, such as Ca^{2+}, Zn, and protein intake, demonstrated the potential to modify associations between Pb and health effects in toxicological studies.

There was suggestive evidence for several other factors as potentially increasing the risk of Pb-related health effects: pre-existing diseases/conditions, stress, and co-exposure with other metals. Pre-existing diseases/conditions have the potential to affect the risk of Pb-related health effects. Recent epidemiologic studies did not support modification of associations between Pb and health endpoints (i.e., mortality, HRV) by the prevalence of diabetes; however, past studies have found individuals with diabetes to be an at-risk population with regard to renal function. Studies of Pb biomarker levels and both renal effects and HRV demonstrated greater odds of the associations among hypertensive individuals compared to those who are normotensive. Stress was evaluated as a factor that potentially increases the risk of Pb-related effects on cognitive function in adults and hypertension and although limited by the small number of epidemiologic studies, increased stress was observed to exacerbate the effects of Pb. Toxicological studies supported this finding. High levels of other metals, such as Cd and Mn, were observed to result in greater effects for the associations between Pb and various health endpoints (e.g., renal function, cognitive function in children) but overall the evidence was limited. Finally, there was inadequate evidence to conclude that smoking, BMI, alcohol

consumption, maternal self-esteem, and cognitive reserve are potential at-risk factors due to limited quantities of studies regarding their effect on Pb-related health outcomes.

1.9.7 Ecological Effects and Corresponding Pb Concentrations

Information on the effects of atmospherically-deposited Pb on some ecosystem receptors in the vicinity of Pb sources is available from studies of terrestrial systems near mining and smelting operations where Pb, as well as other metals, is present in high concentrations. In these studies a decreasing gradient of exposure is observed, and effects in a species or population of interest typically decrease with increasing distance from the source. Thus, concentrations of Pb in moss, lichens and peat have been used to understand spatial and temporal distribution patterns of air Pb concentrations. In other environmental compartments such as sediment and aquatic biota, or in locations not close to Pb sources, evidence that would permit clear attribution of Pb effects to atmospheric deposition is insufficient. Pb that is released into air, soil, or water is then cycled through any or all of these media before reaching an ecological receptor. When a plant, invertebrate, or vertebrate is exposed to Pb, the proportion of observed effects attributable to Pb from atmospheric sources is difficult to assess due to a lack of information not only on deposition, but also on bioavailability, as affected by specific characteristics of the receiving ecosystem and on kinetics of Pb distribution in long-term exposure scenarios. Therefore, the connection between air concentration and ecosystem exposure continues to be poorly characterized for Pb, and the contribution of atmospheric Pb to specific sites is not clear.

Furthermore, the level at which Pb elicits a specific effect is difficult to establish in terrestrial and aquatic systems, due to the influence of other environmental variables on both Pb bioavailability and toxicity, and also to substantial species differences in Pb susceptibility. Current evidence indicates that Pb is bioaccumulated in biota; however, the sources of Pb in biota have only been identified in a few studies, and the relative contribution of Pb from all sources is usually not known. There are large differences in species sensitivity to Pb, and many environmental variables (e.g., pH, organic matter) determine the bioavailability and toxicity of Pb.

1.10 Summary

Table 1-8 characterizes the evidence in the 2006 Pb AQCD (U.S. EPA, 2006b) and previous assessments and compares it to the evidence evaluated in the current assessment. Evidence regarding both the health and ecological effects of Pb are

summarized. The purpose of the table is to highlight the extent to which recent evidence may contribute to current conclusions. The critical assessment of body of evidence as a whole, however, is discussed in Chapter 4 and Chapter 6 of this document, and summarized in Sections 1.6 and 1.7. With regard to ecological effects, evidence pointing to responses in species at ambient or near ambient concentrations is highlighted in the table.

Table 1-8 Summary of evidence from epidemiologic, animal toxicological and ecological studies on the effects associated with exposure to Pb.

Endpoint	Evidence in the 2006 Pb AQCD	Evidence in the 2013 Pb ISA
Health Outcomes:		
Nervous System Effects		
Children		
Cognitive Function in Children	The "overall weight of the available evidence provides clear substantiation of neurocognitive decrements being associated in young children with blood-Pb concentrations in the range of 5-10 µg/dL, and possibly lower." Prenatal, early childhood, lifetime average, and concurrent blood Pb levels were associated with decrements in IQ, learning and executive function. In some cases, concurrent blood Pb level was the strongest predictor.	Recent epidemiologic studies in children continue to demonstrate associations of concurrent blood Pb level with IQ decrements; most recent evidence describes associations of concurrent blood Pb levels with decrements in cognitive abilities related to executive function, and academic performance.
Externalizing Behaviors: Attention, Impulsivity and Hyperactivity in Children	Several epidemiologic studies reported associations between blood and tooth Pb levels and attention decrements in children ages 7-17 years and young adults 19-20 years. Most studies examined prenatal or lifetime average blood Pb levels (means 7, 14 µg/dL) or tooth Pb. The few studies of concurrent blood Pb levels did not find associations with attention in children ages 4-5 years. There were no studies specifically examining ADHD diagnosis. Uncertainty remained regarding whether Pb exposure was an independent predictor of neurobehavioral effects. Prenatal and postnatal Pb exposure was found to reduce ability to inhibit inappropriate responding and increase distractibility in animals.	Recent studies in children continue to support associations of blood Pb levels with attention decrements, impulsivity, and hyperactivity in children ages 7-17 years. A few case-control studies found higher concurrent blood Pb levels in children with diagnosed ADHD; however, because ADHD studies had various limitations,they were not a major consideration in drawing conclusions.
Externalizing Behaviors: Conduct Disorders in Children and Young Adults	Several epidemiologic studies reported associations between Pb exposure and behaviors related to conduct disorders as rated by parents and teachers and criminal offenses in children, adolescents, and young adults. Most studies examined blood Pb levels measured earlier in childhood (means 10-14 µg/dL), tooth Pb, or bone Pb. There was little examination of concurrent blood Pb levels.	Recent studies in children continue to support associations of parent and teacher ratings of behaviors related to conduct disorders with early childhood blood Pb levels and provide new evidence for concurrent blood Pb levels. Additional follow-up of previous cohorts to older ages, support associations of early childhood blood Pb levels or tooth Pb levels with criminal offenses in adults ages 19-24 years.
Internalizing Behaviors in Children	Several prospective studies reported associations of concurrent or childhood average blood, tooth, and bone Pb levels with parent or teacher ratings of withdrawn behavior and symptoms of depression, fearfulness, and anxiety in children ages 3-13 years.	The few recent available studies found associations between concurrent blood Pb level and higher ratings of internalizing behaviors in children ages 8-13 years but these studies had limited consideration for potential confounding.

Table 1-8 (Continued): Summary of evidence from epidemiologic, animal toxicological and ecological studies on the effects associated with exposure to Pb.

Endpoint	Evidence in the 2006 Pb AQCD	Evidence in the 2013 Pb ISA
Visual Function Decrements in Children	The selective action of Pb on retinal rod cells and bipolar cells (e.g., ERG effects) of animals is well documented in earlier AQCDs in animals and found in one study in children.	Additional evidence for retinal effects was found in female rats. High-level developmental Pb exposure did not affect visual acuity in infant monkeys.
Auditory Function Decrements in Childiren	The few available studies reported associations between concurrent blood Pb levels (population means 7-12 µg/dL) and increased hearing thresholds in children. There was coherence with findings in animals but with high Pb exposures (e.g., blood Pb levels 89-150 µg/dL).	The few available recent epidemiologic studies on auditory function in children examined children with high blood Pb levels (means >30 µg/dL) and did not consider potential confounding. Early postnatal Pb exposure of monkeys increased hearing thresholds.
Motor Function Decrements in Children	A small number of studies indicated associations of neonatal, earlier childhood average, lifetime average, and concurrent blood Pb levels (means: 5-12 µg/dL) with poorer fine and motor function in children ages 4.5-17 years. The few toxicological studies did not consistently find Pb-induced impairments in balance and coordination in animals with blood Pb levels >60 µg/dL.	The few recent epidemiologic studies did not consistently find associations between concurrent blood Pb level and decrements in fine motor function. A toxicological study found poorer balance in male mice with gestational plus early postnatal Pb exposure (peak blood Pb level: ≤10 µg/dL).
Adults		
Cognitive Function Decrements in Adults	Among environmentally-exposed adults, bone Pb levels but not blood Pb levels were associated with poorer cognitive performance. These findings point to an effect of long-term cumulative Pb exposure. Impaired learning, memory, and executive function found in adult animals after lifetime Pb exposures beginning in gestation or infancy.	Recent studies support previous findings and recent prospective studies provide new evidence of associations of bone Pb levels with subsequent declines in cognitive function in environmentally-exposed adults over 2-4 year periods.
Psychopathological Effects in Adults	Environmentally-exposed adults were not widely examined; however a study found associations of concurrent blood and tibia Pb level with self-reported symptoms of depression and anxiety in men. Several studies found higher prevalence of symptoms related to mood disorders and anxiety in Pb-exposed workers with mean blood Pb levels 15-38 µg/dL.	Concurrent blood Pb levels were associated with self-reported symptoms of major depressive disorder and general anxiety disorder among men and women participating in NHANES.
Auditory Function Decrements in Adults	A few studies found blood Pb level or cumulative Pb exposure duration to be associated with increased hearing thresholds and hearing loss in Pb-exposed workers.	A prospective study found higher tibia Pb level to be associated with a faster rate of increase in hearing threshold in environmentally-exposed men over a median of 23 years.
Visual Function Decrements in Adults	Decreased visual acuity found in adult monkeys with high blood Pb levels (50-115 µg/dL) after lifetime Pb exposure.	Additional evidence for retinal effects was found in female rats. A case-control study found higher retinal Pb levels in adults with macular degeneration but potential confounding was not considered. Pb-induced effects on ERGs in adult animals vary depending on timing and dose of exposure.

Table 1-8 (Continued): Summary of evidence from epidemiologic, animal toxicological and ecological studies on the effects associated with exposure to Pb.

Endpoint	Evidence in the 2006 Pb AQCD	Evidence in the 2013 Pb ISA
Neurodegenerative Diseases in Adults	In the limited body of epidemiologic studies, occupational Pb exposure and brain Pb levels were not associated with Alzheimer's disease. Blood Pb levels were not consistently associated with Amyotrophic Lateral Sclerosis among environmentally-exposed adults. A few studies found associations between occupational Pb exposure and Parkinson's disease and blood Pb levels and essential tremor. Each study had sufficient limitations. Toxicological studies found Pb-induced neuronal cell death loss and amyloid plaques in aged animals with lactational Pb exposures.	The few case-control studies reported associations of bone Pb levels with Parkinson's disease in environmentally-exposed adults and blood Pb levels with Amyotrophic Lateral Sclerosis and essential tremor. Limitations of previous studies apply to the recent evidence. Recent toxicological evidence suggests that early-life, not adult-only Pb exposure may be associated with neurodegeneration in adult animals.
Cardiovascular Effects		
Hypertension	A meta-analysis of numerous epidemiologic studies estimated that a doubling of blood Pb level (e.g., from 5 to 10 µg/dL) was associated with a 1 mmHg increase in systolic BP and a 0.6 mmHg increase in diastolic BP." Epidemiologic studies consistently demonstrated associations between Pb and incidence of hypertension with suggestive evidence that bone Pb may be associated with hypertension. Animal studies demonstrated that long-term exposure to Pb resulted in hypertension that persisted after cessation of exposure.	Recent epidemiologic and toxicological studies continue to support associations between long-term Pb exposure and increased BP. Recent studies, including those using bone Pb as a metric of cumulative exposure, continue to demonstrate associations of hypertension with Pb levels in adults. Recent studies have emphasized the interaction of cumulative exposure to Pb with other factors including stress.
Subclinical Atherosclerosis	One NHANES analysis reported an association of blood Pb with PAD	Limited evidence for Pb-induced subclinical atherosclerosis, including one high-quality epidemiologic study that reports an increasing trend in the odds of PAD and concurrent blood Pb level in adults. Recent toxicological studies describe a plausible biological mechanism.
Coronary Heart Disease	The evidence for an association of Pb with cardiovascular mortality was limited but supportive. A few cross-sectional studies indicated associations between Pb biomarker levels and increased risk of CHD outcomes (i.e., MI and left ventricular hypertrophy).	Recent studies address limitations of previous studies and provide additional evidence for an association of Pb with cardiovascular mortality in adults. Specific causes of mortality that were associated with Pb could be related to increased BP and hypertension.
Cerebrovascular Disease	Evidence available on the risk of cerebrovascular disease from Pb exposure was limited to one study of stroke.	Evidence for increased risk of mortality from stroke is inconsistent.

Table 1-8 (Continued): Summary of evidence from epidemiologic, animal toxicological and ecological studies on the effects associated with exposure to Pb.

Endpoint	Evidence in the 2006 Pb AQCD	Evidence in the 2013 Pb ISA
Renal Effects		
Reduced Kidney Function	Circulating and cumulative Pb was associated with longitudinal decline in renal function in adults. Toxicological studies demonstrated that initial accumulation of absorbed Pb occurred primarily in the kidneys and noted a hyperfiltration phenomenon during the first 3 months of exposure, followed by decrements in kidney function.	Recent epidemiologic and toxicological studies add to the body of evidence on Pb exposure and kidney dysfunction (e.g., lower creatinine clearance, higher serum creatinine, and lower GFR) in nonoccupationally-exposed adults.
Immune System Effects		
Increases in Atopic and Inflammatory Conditions	Children:	
	Several epidemiologic studies suggested that Pb exposure may be associated with effects on cellular and humoral immunity in children. The principal effect demonstrated increases in serum immunoglobulin E (IgE) levels with concurrent blood Pb levels >10 µg/dL. Toxicological evidence supported these findings with extensive evidence for prenatal and early postnatal Pb exposures skewing toward Th2 cytokine production and affecting downstream events such as increases in IgE and inflammation Several toxicological studies found a Pb-induced shift to Th2 cytokine production and a hyperinflammatory phenotype of macrophages in animals with long-term (>4 weeks) prenatal or postnatal Pb exposure.	Recent studies in children added to the evidence for associations of blood Pb levels with asthma, allergy, and IgE. The consistency and coherence of findings among related immune effects that support a shift from a Th1 to a Th2 phenotype supports the biological plausibility for epidemiologic observations of associations with asthma, allergy and inflammation-related effects in other organ systems.
	Adults:	
	Pb exposure-associated immune effects were not widely examined in environmentally-exposed adults.	A small body of recently available studies provide new evidence for increases in cytokines and other indicators of inflammation in association with higher concurrent blood Pb level. A few available toxicological studies find Pb-associated increases in cytokines and effects on dendritic cells in adult mice.

Table 1-8 (Continued): Summary of evidence from epidemiologic, animal toxicological and ecological studies on the effects associated with exposure to Pb.

Endpoint	Evidence in the 2006 Pb AQCD	Evidence in the 2013 Pb ISA
Decreases in Host Resistance	Toxicological evidence demonstrated Pb-induced increases in bacterial and viral infection and suppressed DTH in animals. These effects were supported by extensive evidence for prenatal and early postnatal Pb exposures decreasing Th1 cytokine production, for short-term prenatal Pb exposure decreasing nitric oxide production by macrophages), and for long-term (>4 weeks) exposure Pb exposure inducing a hyperinflammatory phenotype of macrophages in adult animals. A few epidemiologic studies found higher prevalence of respiratory infections in association with higher blood Pb levels in children and occupational Pb exposure in adults; however, studies did not consider potential confounding. In the large body of studies in occupationally-exposed adults, the most consistent findings were reduced neutrophil functionality in workers with blood Pb levels >14.8-91 µg/dL. Environmentally-exposed adults were not widely examined.	A small body of recent studies supports previous findings of decreased bacterial resistance and decreased IFN-γTh1 cytokine production in animals. Epidemiologic evidence is limited to an ecological study of soil and lichen Pb that lacked consideration for potential confounding.
Autoimmunity	A small number of toxicological studies found that prenatal and postnatal Pb treatment, several by i.p. injection, increased generation of auto-antibodies. A study found higher auto-antibodies to neural proteins in Pb-exposed workers with blood Pb levels 10-40 µg/dL	A recent toxicological study provided indirect evidence by showing Pb-induced increases in the activation of neo-antigen specific T cells, which have the potential to induce formation of auto-antibodies.
Hematologic System		
Red Blood Cell Function and Heme Synthesis	Children:	
	Pb exposure was associated with disruption in heme synthesis with increases in blood Pb levels of approximately 20 µg/dL sufficient to halve ALAD activity and inhibit ferrochelatase. Risk of clinical anemia in children becomes apparent at high blood Pb levels: 10% probability of anemia was estimated to be associated with ~20 µg/dL Pb at 1 year of age, 50 µg/dL at 3 years of age, and 75 µg/dL at 5 years of age.	Recent epidemiologic studies provide evidence that exposure to Pb is associated with numerous deleterious effects on the hematological system in children, including altered hematological parameters (Hb, MCV, MCH, RBC count), perturbed heme synthesis mediated through decreased ALAD and ferrochelatase activities, and oxidative stress.
	Adults:	
	Pb exposure was associated with disruption in heme synthesis with increases in blood Pb levels of approximately 20 µg/dL sufficient to halve ALAD activity and inhibit ferrochelatase. Exposures to Pb resulting in blood concentrations <40 µg/dL appear to be tolerated without decreases in blood hemoglobin or hematocrit, however changes in erythropoiesis do occur at these blood levels.	Recent epidemiologic studies provide evidence exposure to Pb is associated with numerous deleterious effects on the hematological system in adults, including altered hematological parameters (Hb, MCV, MCH, RBC count), perturbed heme synthesis mediated through decreased ALAD and ferrochelatase activities, decreased erythropoiesis, and oxidative stress.

Table 1-8 (Continued): Summary of evidence from epidemiologic, animal toxicological and ecological studies on the effects associated with exposure to Pb.

Endpoint	Evidence in the 2006 Pb AQCD	Evidence in the 2013 Pb ISA
Developmental and Reproductive Effects		
Development	Epidemiologic studies reported effects including delayed puberty in girls. Animal toxicological studies reported Pb-associated developmental effects on teeth, sensory organs, the GI system, the liver, and postnatal growth. Delayed puberty was also observed in both male and female populations in animal toxicology studies showing associations with dam blood Pb levels of ~40 µg/dL and pup blood Pb levels of 26 µg/dL.	Recent toxicological and epidemiologic studies provide evidence for delayed onset of puberty in males and females. Most studies found delayed onset of puberty was among children ages 6-18 years. These findings were supported by studies in the animal toxicological literature showing effects on puberty onset at blood Pb levels of 3.5-13 µg/dL.
Birth Outcomes	Toxicological studies reviewed concluded that Pb exposure can increase fetal mortality and produce sublethal effects, smaller litters, reduced birth weight, and fewer implantation sites. Epidemiologic studies on preterm birth and low birth weight/fetal growth reported inconsistent findings. Epidemiologic studies reported the possibility of small associations between increased Pb exposure and birth defects, and toxicological studies demonstrated associations between exposure to high doses of Pb and increased incidences of teratogenic effects.	Recent toxicological and epidemiologic studies have reported inconsistent findings for studies for birth defects, preterm birth, and low birth weight/fetal growth. A few well-conducted epidemiologic studies of preterm birth and low birth weight/fetal growth reported associations between increased Pb levels and decreased gestational age and birth weight/fetal growth.
Male Reproductive Function	Epidemiologic evidence suggested small associations between Pb exposure and male reproductive outcomes including perturbed semen quality and increased time to pregnancy. Associations between Pb exposure and male reproductive endocrine status were not observed in the occupational populations studied. Toxicological studies provided evidence that Pb produced effects on male and female reproductive junction and development and disrupts endocrine function.	Recent toxicological studies provide strong evidence for effects on sperm (blood Pb levels 5-43 µg/dL). Epidemiologic studies support the association observed in toxicological studies of Pb exposure and detrimental effects on sperm.
Female Reproductive Function	Toxicological studies reported that Pb exposure was associated with effects on female reproductive function that can be classified as alterations in female sexual maturation, effects on fertility and menstrual cycle, endocrine disruption, and changes in morphology or histology of female reproductive organs including the placenta. Epidemiologic studies on Pb and female reproductive function provided little evidence for an association between Pb biomarkers and effects on female reproduction and fertility.	Epidemiologic studies of Pb levels and hormones demonstrate associations but are inconsistent overall and there is a lack of large, well-conducted epidemiologic studies examining associations between Pb levels and fertility. Toxicological studies of Pb and effects on female reproduction demonstrate effects in some studies.

Table 1-8 (Continued): Summary of evidence from epidemiologic, animal toxicological and ecological studies on the effects associated with exposure to Pb.

Endpoint	Evidence in the 2006 Pb AQCD	Evidence in the 2013 Pb ISA
Cancer		
Cancer	Epidemiologic studies of highly exposed occupational populations suggest a relationship between Pb and cancers of the lung and the stomach; however the evidence is limited by the presence of various potential confounders, including metal co-exposures (e.g., to As, Cd), smoking, and dietary habits. The 2003 NTP and 2004 IARC reviews concluded that Pb and Pb compounds were probable carcinogens, based on limited evidence in humans and sufficient evidence in animals. Based on animal data and inadequate human data Pb and Pb compounds would be classified as likely carcinogens according to the EPA Cancer Assessment Guidelines for Carcinogen Risk Assessment.	The toxicological literature continues to provide the strongest evidence for Pb exposure and cancer with supporting evidence provided by the epidemiologic literature. Epidemiologic studies of cancer incidence and mortality reported inconsistent results.

Ecological/Welfare Effects:		
Endpoint	**Evidence in the 2006 Pb AQCD**	**Evidence in the 2013 Pb ISA**
Developmental and Reproductive Effects	Terrestrial Organisms:	
	No information on reproduction in plants.	There is an insufficient number of studies that consider Pb effects on plant reproduction.
	Limited evidence in invertebrates and vertebrates.	Recent studies in a few taxa expand the evidence for Pb effects on developmental and reproductive endpoints for invertebrates and vertebrates at concentrations that generally exceed Pb levels in U.S. soils. In some organisms, exposure-dependent responses in development and reproductive outcomes are observed in experiments where exposure increases from background concentrations to concentrations found in heavily exposed sites near stationary sources. Data on terrestrial species is coherent with toxicological data from mammals in the context of human health research.
	Aquatic Organisms:	
	No reviewed studies on reproductive effects in aquatic plants. Reproductive and developmental effects reported in a few species of invertebrates at <50 µg Pb/L and in fish at <150 µg Pb/L	Recent evidence supports previous findings of reproductive and developmental effects of Pb in freshwater invertebrates and vertebrates and differential lifestage response at near ambient concentrations of Pb in some organisms. For saltwater invertebrates there is limited evidence for effects on early development at Pb concentrations higher than typically detected in marine environments.

Table 1-8 (Continued): Summary of evidence from epidemiologic, animal toxicological and ecological studies on the effects associated with exposure to Pb.

Endpoint	Evidence in the 2006 Pb AQCD	Evidence in the 2013 Pb ISA
Growth	Terrestrial Organisms:	
	Pb inhibits photosynthesis and respiration in plants. Limited evidence for growth effects in soil invertebrates, avian and mammalian consumers.	Recent studies support previous findings of Pb effects on plant growth, with some evidence for exposure-dependent decreases in the biomass of some plant species grown in Pb-amended or Pb-contaminated soil. Recent data for soil invertebrates supports previous evidence of increasing effects on growth with increasing exposure. Limited studies considered effects on growth on vertebrates.
	Aquatic Organisms:	
	Evidence for growth effects in algae, aquatic plants and aquatic invertebrates Most primary producers experience EC_{50} values for growth in the range of 1,000 to 100,000 µg Pb/L	The weight of the evidence continues to support growth effects of Pb in freshwater plants and invertebrates. Recent studies on growth in freshwater invertebrates find effects of Pb at lower concentrations than previously reported. Growth inhibition in one species of freshwater snail was observed at <4 µg Pb/L in juveniles. Lowest genus mean chronic value for Pb reported at 10 µg Pb/L in a freshwater mussel.
Survival	Terrestrial Organisms:	
	No information on mortality in plants. Effects of Pb on invertebrates and vertebrates include decreased survival. In terrestrial and avian species toxicity was observed in laboratory studies over a wide range of doses (<1 to >1,000 mg Pb/kg body weight per day) (U.S. EPA, 2005b).	Recent studies in invertebrates and vertebrates support previous associations between Pb exposure and mortality.
	Aquatic Organisms:	
	No studies reviewed on mortality in plants at current concentrations of Pb in the environment. Pb impacted survival of some aquatic invertebrates at <20 µg Pb/L dependent upon water quality variables (i.e., DOC, hardness, pH). Range of 96-hour LC_{50} values in fathead minnow: 810->5,400 µg Pb/L	The weight of evidence continues to support Pb effects on survival of freshwater invertebrates and vertebrates and indicates that there are effects in a few species at lower concentrations than previously reported. Recent evidence for effects in a few freshwater invertebrates at <20 µg Pb/L Recent evidence in freshwater fish for impacts to survival at <100 µg Pb/L dependent upon water quality parameters and lifestage. 96- hour LC_{50} values as low as 41 µg Pb/L in fathead minnows tested in natural waters from across the U.S.

Table 1-8 (Continued): Summary of evidence from epidemiologic, animal toxicological and ecological studies on the effects associated with exposure to Pb.

Endpoint	Evidence in the 2006 Pb AQCD	Evidence in the 2013 Pb ISA
Neurobehavioral Effects	Terrestrial Organisms:	
	Exposure to Pb in laboratory studies and simulated ecosystems may alter species competitive behaviors, predator-prey interactions, and contaminant avoidance behaviors.	Recent studies continue to support previous evidence that Pb exposure is associated with behavioral alterations. Recent studies identify possible molecular targets for Pb neurotoxicity in invertebrates and there is new evidence in a few invertebrate and vertebrate species for behavioral effects associated with Pb exposure (i.e., feeding and escape behaviors).
	Aquatic Organisms:	
	Exposure to Pb has been shown to affect brain receptors in fish and may alter avoidance behaviors and predator-prey interactions.	Recent studies continue to support previous evidence that Pb exposure is associated with behavioral alterations. Recent studies identify possible molecular targets for Pb neurotoxicity in fish and provide additional evidence for Pb effects on behaviors in freshwater organisms that may impact predator avoidance (swimming).
Hematological Effects	Terrestrial Organisms:	
	Pb effects on heme synthesis were documented in the 1986 Pb AQCD and continue to be studied in terrestrial biota. Changes in ALAD are not always related to adverse effects but may simply indicate exposure. The linkage between effects of Pb on blood parameters is well documented; however, the linkage between hematological indicators and ecologically relevant effects is less well understood.	Consistent with previous studies, the weight of the evidence in recent studies continues to support findings of Pb effects on heme synthesis and ALAD enzyme activity. Recent studies in birds near historical mining areas and altered serum profiles and blood cell counts in vertebrates provide evidence for additional species in which hematological endpoints are potentially affected by Pb.
	Aquatic Organisms:	
	In metal impacted habitats, ALAD is a recognized biomarker of Pb exposure. Changes in ALAD are not always related to adverse effects but may simply indicate exposure. In fish, Pb effects on blood chemistry have been documented with Pb concentrations ranging from 100 to 10,000 µg Pb/L.	Consistent with previous studies, the weight of the evidence in recent studies continues to support findings of Pb effects on ALAD and expands this evidence to additional species of bacteria, invertebrates, and vertebrates as well as in recent studies on altered blood cell counts in vertebrates. Additional field studies in both fresh and saltwater bivalves report a correlation between Pb and ALAD activity.

Table 1-8 (Continued): Summary of evidence from epidemiologic, animal toxicological and ecological studies on the effects associated with exposure to Pb.

Endpoint	Evidence in the 2006 Pb AQCD	Evidence in the 2013 Pb ISA
Physiological Stress	Terrestrial Organisms:	
	Pb exposure may cause lipid peroxidation and changes in glutathione concentrations. There are species differences in resistance to oxidative stress.	Recent studies continue to support previous associations of Pb exposure with physiological stress. New evidence includes upregulation of antioxidant enzymes, production of reactive oxygen species and increased lipid peroxidation associated with Pb exposure in additional species of terrestrial plants, invertebrates and vertebrates. Increasing effects follow increasing experimental exposures from background concentrations to concentrations found in heavily exposed sites near stationary sources.
	Aquatic Organisms:	
	Pb exposure associated with alterations in enzymes involved in physiological stress responses.	Recent studies continue to support previous associations of Pb exposure with physiological stress. New evidence in freshwater organisms includes upregulation of antioxidant enzymes, production of reactive oxygen species and increased lipid peroxidation associated with Pb exposure. Changes in antioxidant activity are reported in some saltwater invertebrates. Observed effects generally occurred at concentrations that typically exceed Pb levels in U.S. waters with limited evidence for effects associated with Pb at polluted sites.

Table 1-8 (Continued): Summary of evidence from epidemiologic, animal toxicological and ecological studies on the effects associated with exposure to Pb.

Endpoint	Evidence in the 2006 Pb AQCD	Evidence in the 2013 Pb ISA
Community and Ecosystem Level Effects	Terrestrial Ecosystems:	
	Effects of Pb difficult to interpret because of the presence of other stressors including metals. The 1986 Pb AQCD reported shifts toward Pb-tolerant communities at 500 to 1,000 mg Pb/kg soil. In the 2006 Pb AQCD, decreased species diversity and changes in community composition were observed in ecosystems surrounding former smelters.	Recent evidence for effects of Pb in soil microbial communities adds to the body of evidence for effects at higher levels of biological organization. In addition, effects of Pb uptake on reproduction, growth, and survival at the species level are likely to lead to effects at the population, community, and ecosystem level. However, most evidence for Pb toxicity to terrestrial biota is from single-species assays, and there are important uncertainties in generalizing from effects observed under small-scale, controlled conditions, up to effects at the ecosystem level of biological organization.
	Aquatic Ecosystems:	
	Most evidence of community and ecosystem level effects is from near Pb sources, usually mining effluents. Effects of Pb difficult to interpret because of the presence of other stressors including metals. Generally, there is insufficient information available for single materials in controlled studies to permit evaluation of specific impacts on higher levels of organization (beyond the individual organism).	Recent evidence for Pb effects on sediment-associated and freshwater aquatic plant communities add to the body of evidence of effects at higher levels of biological organization. However, most evidence for Pb toxicity to aquatic biota is from single-species assays. Uncertainties exist in generalizing effects observed under small-scale, predicted conditions up to effects at the ecosystem-level however, uptake of Pb into aquatic organisms and subsequent effects on reproduction, growth, and survival at the species level are likely to lead to effects at the population, community, and ecosystem level.

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CHAPTER 2 AMBIENT LEAD: SOURCE TO CONCENTRATION

2.1 Introduction

This chapter reviews concepts and findings in atmospheric sciences that provide a foundation for the detailed presentation of evidence of Pb exposure and Pb-related health and ecological effects in subsequent chapters. Information in this chapter builds on previous Pb AQCDs using more recent data and studies. This includes new knowledge of Pb fate and transport, the latest developments in monitoring and analysis methodologies, and recent data describing Pb concentrations as a function of size range. The chapter focuses on Pb concentrations in the U.S. but includes non-U.S. studies to the extent that they are informative regarding current conditions in the U.S. Description of the chemical forms of Pb is not provided here, however, because this information is well established. The reader is referred to the 2006 Pb AQCD for a description of the chemical forms of Pb (U.S. EPA, 2006b).

Section 2.2 provides an overview of the sources of ambient air Pb. Section 2.3 provides a description of the fate and transport of Pb in air, soil, and aqueous media. Descriptions of Pb measurement methods, monitor siting requirements, and monitor locations are presented in Section 2.4. Ambient Pb concentrations, their spatial and temporal variability, size distributions of Pb-bearing particulate matter (PM), associations with copollutants and background Pb concentrations are characterized in Section 2.5. Concentrations of Pb in non-air media and biota are described in Section 2.6.

2.2 Sources of Atmospheric Pb

The following section reviews emissions estimates from the 2008 National Emissions Inventory (NEI), version 3 (U.S. EPA, 2013), updated with EPA's best estimates of piston-engine aircraft emissions[1]. This section also reviews updated information from the peer-reviewed literature regarding sources of ambient Pb. Detailed information about processes for anthropogenic emissions and naturally-occurring emissions can be found in the 2006 Pb AQCD (U.S. EPA, 2006b). The papers cited herein generally utilized PM sampling data, because a majority of ambient airborne Pb readily condenses to PM. The mobile source category included combustion products from organic Pb antiknock

[1] The piston-engine aircraft emissions inventory can be obtained from the following site: http://www.epa.gov/ttn/chief/net/2008neiv2/2008_neiv2_tsd_draft.pdf.

additives used in piston-engine aircraft (hereafter referred to piston-engine aircraft emissions).

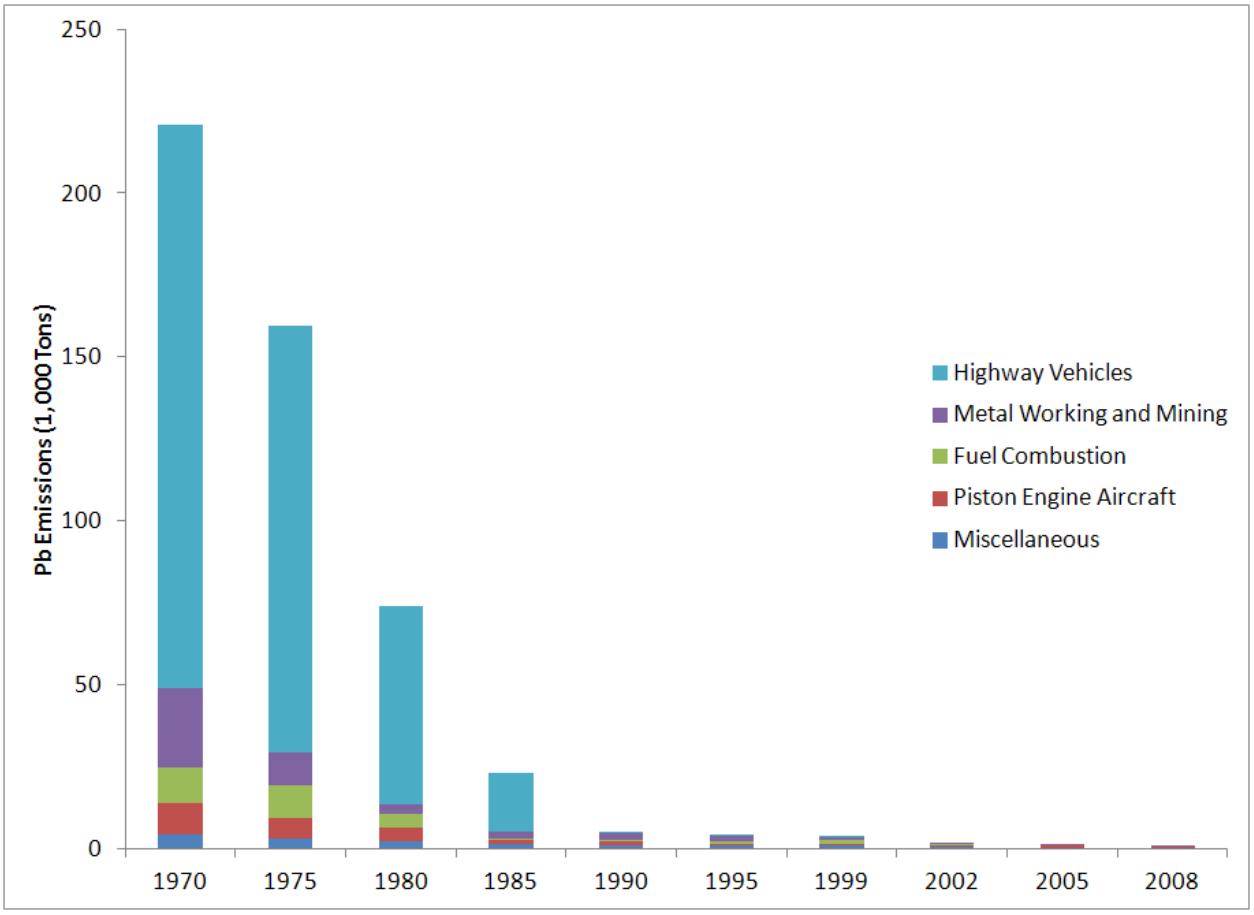
2.2.1 National Emissions Inventory

The 2006 Pb AQCD (U.S. EPA, 2006b) listed the largest sources to be (in order): industrial-commercial-institutional boilers and process heaters (17%), coal utilities boilers (12%), mobile sources (10%), iron and steel foundries (8%), and miscellaneous sources from industrial processes, incineration, and utilities, each contributing less than 5% (53%). The sources listed in the 2006 Pb AQCD were based on the 2002 NEI (U.S. EPA, 2006a). Subsequent correction of computational errors prior to completion of the 2008 NAAQS review provided corrected estimates for the 2002 inventory which indicated the largest sources to be (in order): mobile sources from leaded aviation gas usage in piston-engine aircraft (45%), metallurgical industries (23%), manufacturing (14%), incineration (8%), boilers (6%), and miscellaneous sources contributing less than 5% (U.S. EPA, 2007h). The 2002 and prior year inventories discussed in this document reflect the corrected information.

Emissions of Pb have dropped substantially over the past forty years, as shown in Figure 2-1 and Figure 2-2. The reduction before 1990 is largely due to the phase-out of Pb as an anti-knock agent in gasoline for on-road automobiles, as discussed in the 2006 Pb AQCD (U.S. EPA, 2006b). This action resulted in a 98% reduction in Pb emissions from 1970-1995. Total Pb emissions over the period 1995-2008 decreased an additional 77%, from 4,100 tons in 1995 to 950 tons in 2008. Additional emissions reductions are related to enhanced control of the metals processing industry. In 1995, metals processing accounted for 42% (2,200 tons) of total Pb emissions. By 2008, metals processing accounted for 19% (178 tons) of total emissions. This represented more than an order of magnitude decrease in Pb emissions from metals processing. Emissions from piston-engine aircraft decreased 34% over this time period. In 1990, nonroad Pb emissions were 990 tons, 830 tons of which were generated from piston-engine aircraft, and represented 19% of total Pb emissions. In 2008, nonroad Pb emissions from piston-engine aircraft were slightly lower at 550 tons,[1] which represented 58% of all Pb emissions. 2008 piston-engine aircraft emissions were comprised of 254 tons of Pb from emissions at or near airports and 296 tons of Pb emitted in flight (i.e., outside the landing and take-off cycles). "Miscellaneous" emissions from other industrial processes, solvent utilization, agriculture, and construction constituted 11% of emissions (103 tons, 80 of which were

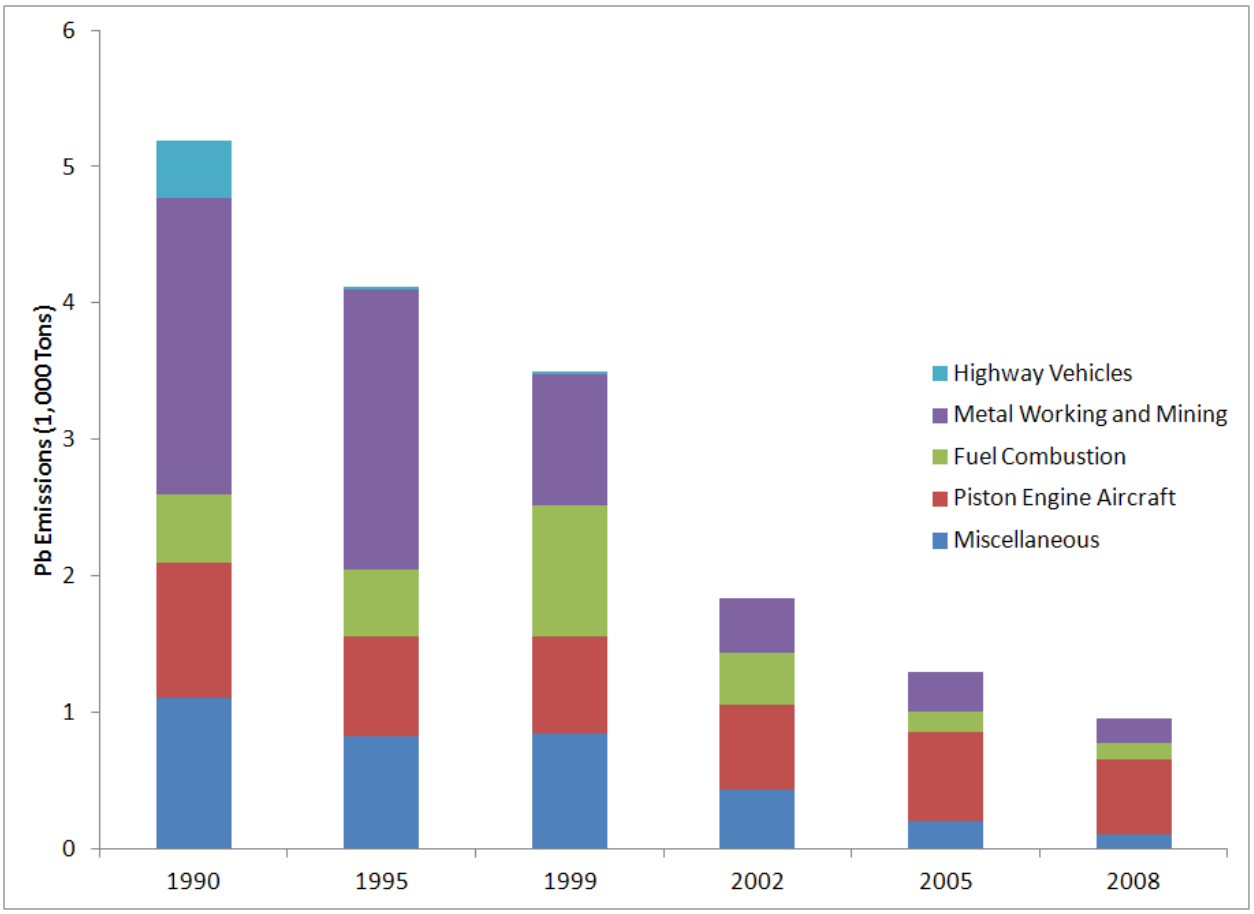
[1] This reflects EPA's best estimates of piston-engine aircraft emissions. The piston-engine aircraft emissions inventory can be obtained from the following site: http://www.epa.gov/ttn/chief/net/2008neiv2/2008_neiv2_tsd_draft.pdf.

from industrial sources other than metal working and mining) in 2008 (U.S. EPA, 2011a, 2008a).



Source: U.S. EPA (2011a, 2008a)

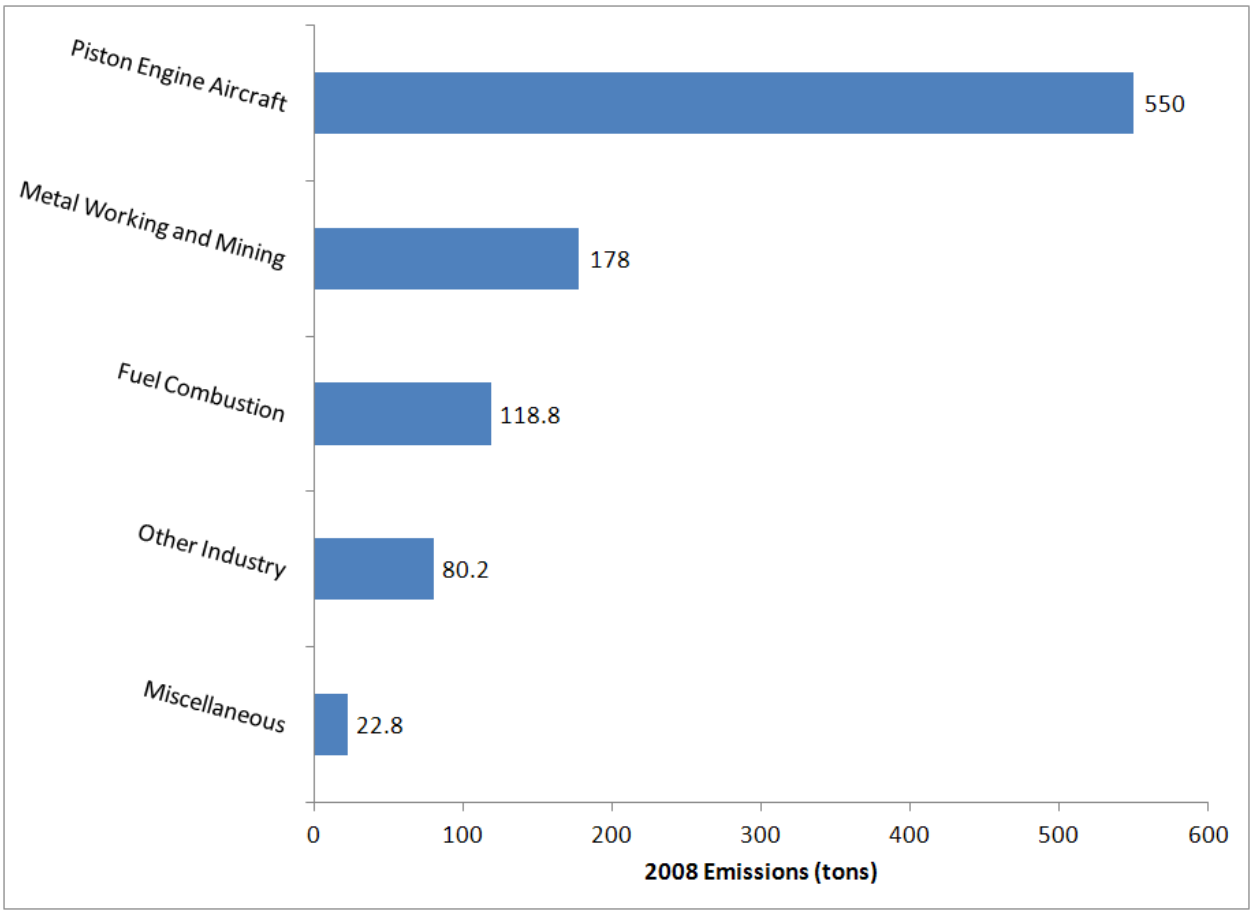
Figure 2-1 Trends in Pb emissions (thousand tons) from stationary and mobile sources in the U.S., 1970-2008.



Source: U.S. EPA (2011a, 2008a)

Figure 2-2 Trends in Pb emissions (thousand tons) from stationary and mobile sources in the U.S., 1990-2008.

Direct emissions of Pb into the atmosphere primarily come from piston-engine aircraft, fuel combustion, and industrial activities. Direct Pb emissions estimated by the 2008 NEI are shown in Figure 2-3. Piston-engine aircraft produced 58% of all emissions (550 tons). Metal working and mining contributed 178 tons (19%) of Pb emissions in 2008, followed by fuel combustion (13%), other industry (8%), and miscellaneous contributions from agriculture, solvent utilization, and operation of commercial marine vessels and locomotives (2%) (U.S. EPA, 2011a). Pb emissions from the "metal working and mining" category include the single primary Pb smelter in the U.S., the Doe Run facility in Herculaneum, MO; secondary Pb smelters, mostly designed to reclaim Pb for use in Pb-acid batteries; and smelters for other metals.

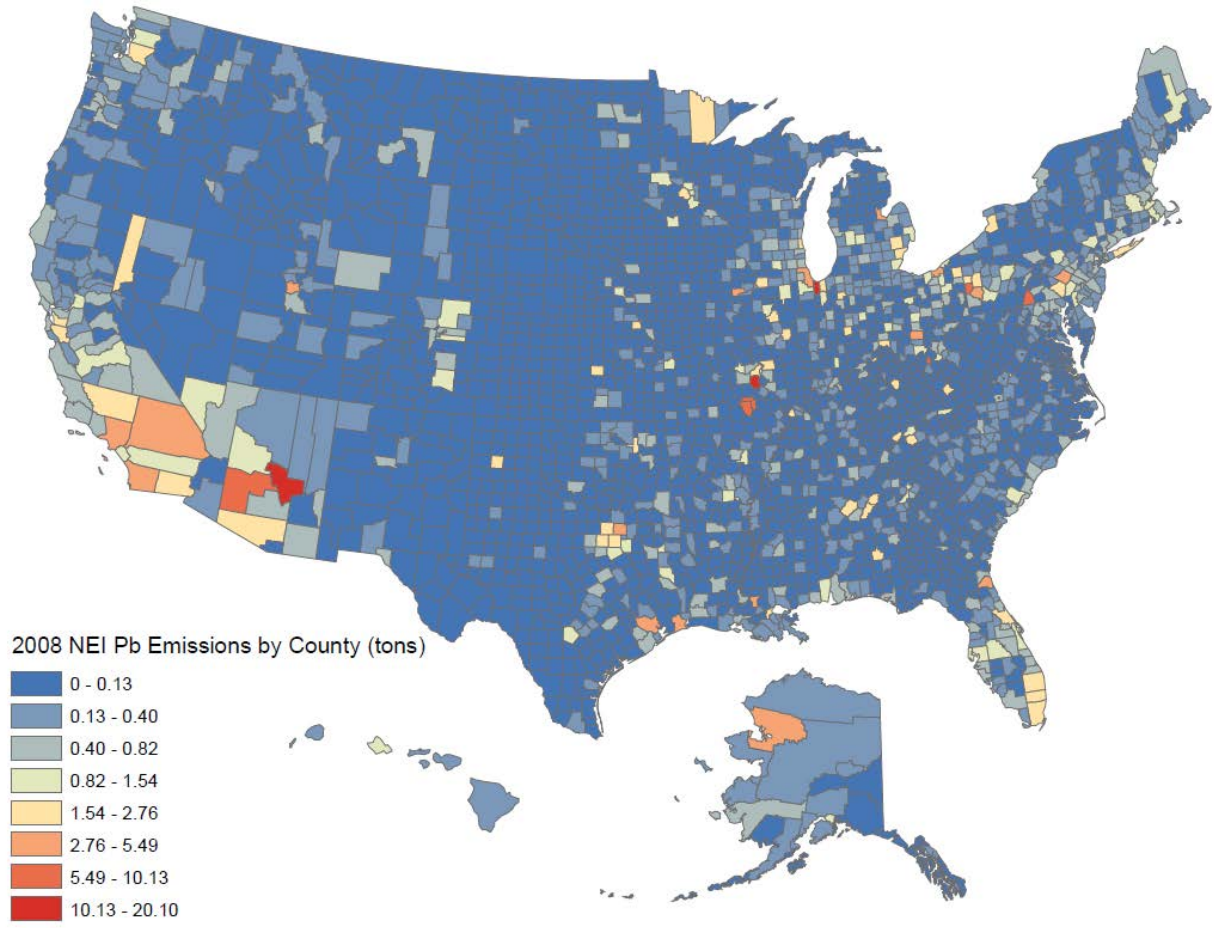


Source: U.S. EPA (2011a)

Figure 2-3 Nationwide stationary and mobile source Pb emissions (tons) in the U.S. by source sector in 2008.

There is substantial variability in Pb emissions across U.S. counties, as shown in Figure 2-4 for the continental U.S. The emissions levels, shown in units of tons, vary over several orders of magnitude. Ninety-five percent of U.S. counties, territories, and tribal areas had 2008 emissions below 1 ton; 50% of counties, territories, and tribal areas had 2008 emissions below 0.042 tons. Jefferson County, MO was the highest emitting single county, with over 20 tons of airborne Pb emissions in 2008. Jefferson County is home to the Doe Run primary Pb smelting facility, which is the only remaining operational primary Pb smelter in the U.S. and is planning to cease the existing smelter operations at this site by April, 2014 (DRRC, 2010). Pb emissions from piston-engine aircraft operating on leaded fuel are estimated to occur at approximately 20,000 airports across the U.S. Many of the more active airports are more numerous in highly populated metropolitan regions, which suggests that emissions from piston-engine aircraft may be higher in these locations compared with rural areas. In twenty-five counties, piston-

engine aircraft are estimated to emit cumulatively greater than one ton of Pb in 2008 U.S. EPA (2011a).



Source: U.S. EPA (2011a)

Figure 2-4 County-level Pb emissions (tons) in the U.S. in 2008.

Figure 2-5 illustrates the locations and relative magnitude of Pb emissions for 124 facilities in the U.S. emitting 0.5 tons or more in 2008 (U.S. EPA, 2011a). One facility, Doe Run in Herculaneum (Jefferson Co.), MO, emitted more than 10 tons in 2008. Additionally, the map illustrates several locations where there is a confluence of point sources (not to be confused with total sources including non-point), including Jefferson Co., MO, Lake Co., IN, Iron Co., MO, and Gila, AZ, in each of which are facilities that were estimated to cumulatively emit between 5 and 10 tons. Among the facilities shown, 124 are non-airport facilities; eight of these were estimated to emit 5 tons or more, 53 to emit between 1 and 5 tons, and 63 to emit between 0.5 and 1 tons in 2008. Figure 2-5

additionally includes 58 airports, the six largest of which were estimated to emit between 1 and 5 tons.

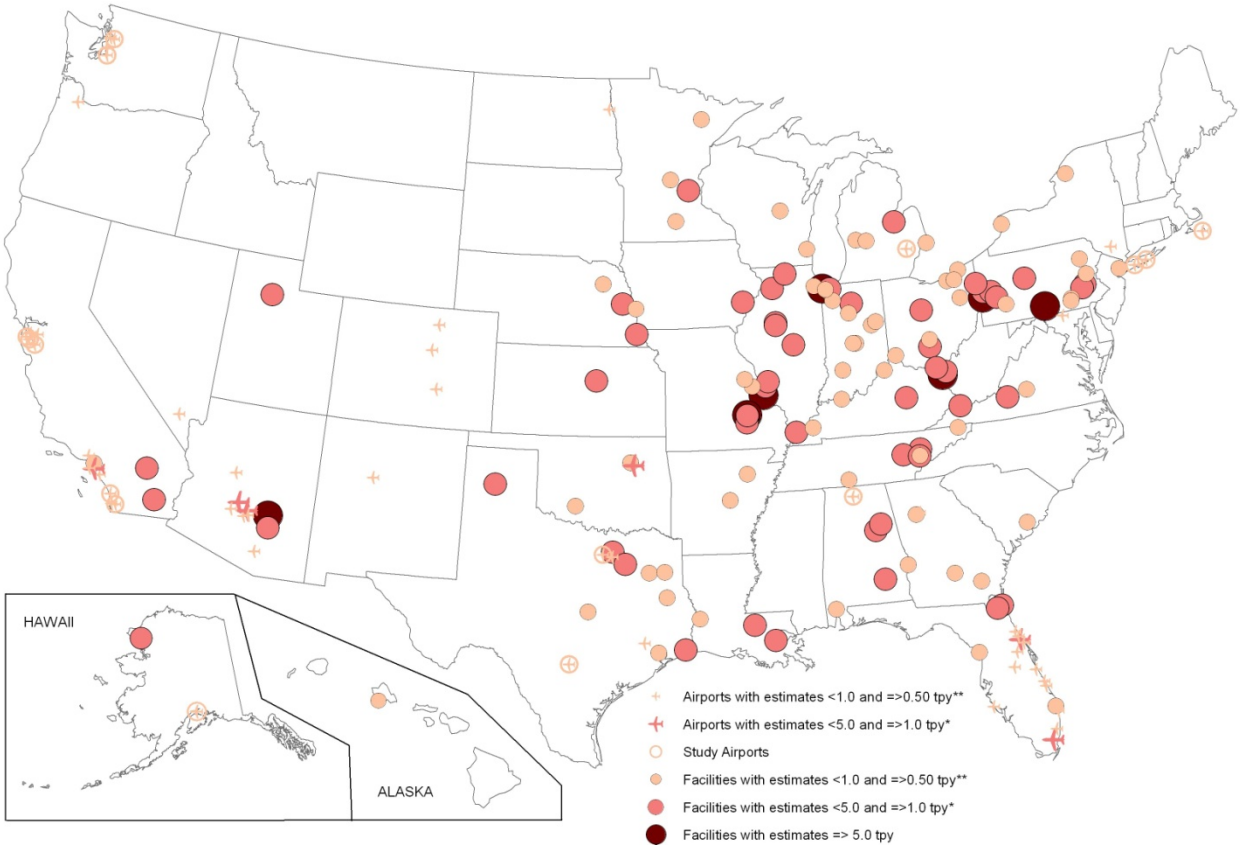


Figure 2-5 Pb facilities estimated to emit 0.5 tons or more in 2008.

2.2.2 Anthropogenic Sources

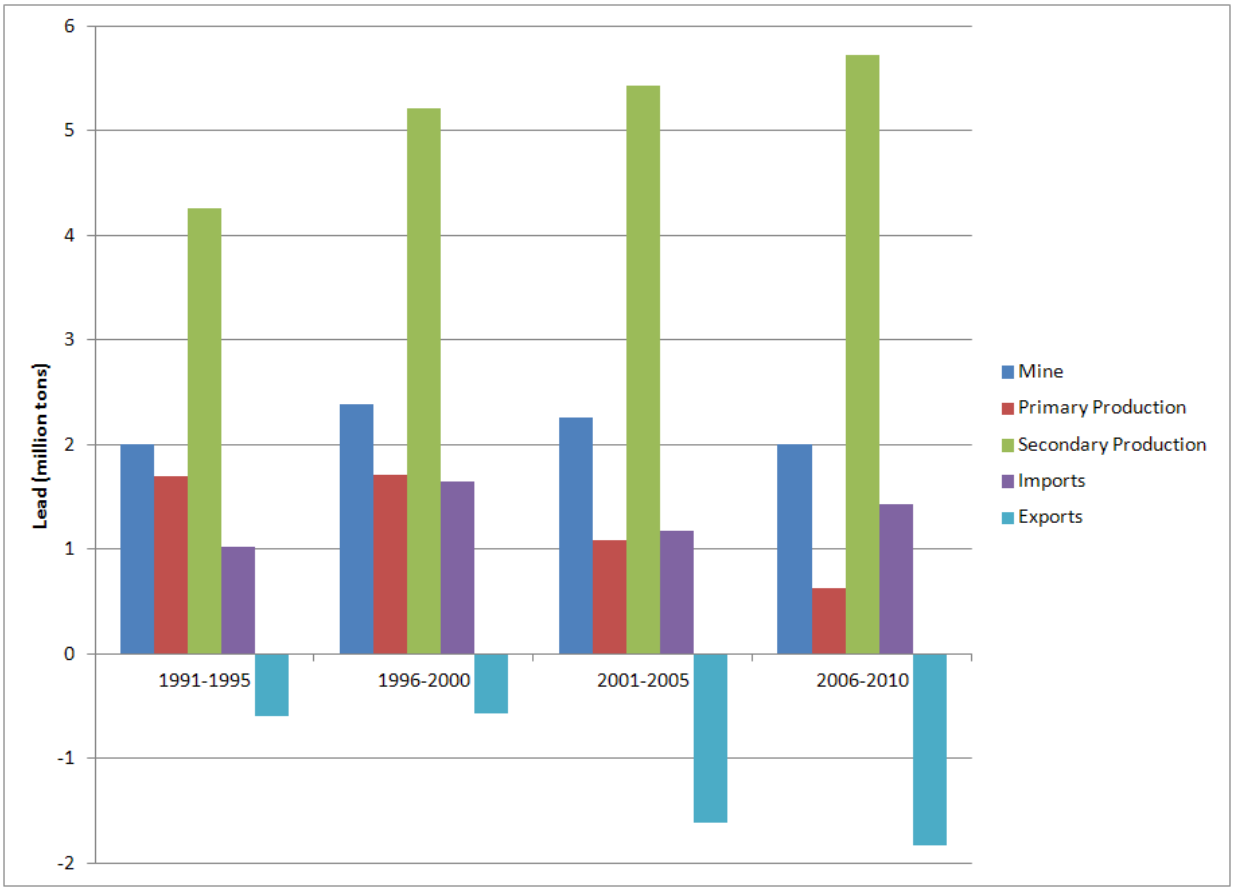
Anthropogenic Pb source categories are organized below in order of magnitude with regard to the sum of emissions nationally reported on the 2008 NEI (U.S. EPA, 2011a). Pb sources were reviewed in the 2006 Pb AQCD (U.S. EPA, 2006b) by species. Forms of Pb commonly observed in the environment are carried forward from the 2006 Pb AQCD (U.S. EPA, 2006b) and are presented in Table 2-1 to serve as a reference for the categories of Pb sources described in Sections 2.2.1 and 2.2.2.

Table 2-1 Pb compounds observed in the environment.

Emission Source	Observed Pb Compounds
Minerals	PbS (Galena)
	PbO (Litharge, Massicot)
	Pb_3O_4 ("Red Pb")
	$PbSO_4$ (Anglesite)
Smelting aerosols	Pb^0, PbS
	$PbSO_4$, PbO
	$PbCO_3$
	Pb silicates
Coal combustion aerosols	PbS
	PbSe
Coal combustion flue gases	Pb^0, PbO, PbO_2 (Above 1,150 K)
	$PbCl_2$ (Low rank coals, above 1,150 K)
	$PbSO_4$ (Below 1,150 K)
Wood combustion	$PbCO_3$
Waste incineration aerosols	$PbCl_2$, PbO
Soils near mining operations	$PbCO_3$
	$PbSO_4$
	$[PbFe_6(SO_4)_4(OH)_{12}]$
	$[Pb_5(PO_4)_3Cl]$
	$[Pb_4SO_4(CO_3)_2(OH)_3]$
	$PbS\text{-}Bi_2S_3$
	Pb oxides, silicates
Piston-engine aircraft emissions, racing vehicle exhaust (combustion of leaded fuel)	$PbBr_2$
	Alkyl Pb
	$PbBrCl\text{-}NH_4Cl$, $PbBrCl\text{-}2NH_4Cl$
Roadside dust	$PbSO_4$, Pb^0, $PbSO_4(NH_4)SO_4$, Pb_3O_4, $PbO\text{-}PbSO_4$ and $_2PbCO_3\text{-}Pb(OH)_2$
Brake wear, wheel weights	Pb^0
Aircraft engine wear	Pb^0

Source: Biggins and Harrison (1980, 1979); U.S. EPA (2006b).

Pb emissions in the U.S. derive from a combination of mined, processed, and imported Pb. Figure 2-6 illustrates trends in the origin of Pb used in various sectors described below. Over the period 1991-2010, the amount of Pb used in secondary Pb processing increased by 37%. Exports of Pb increased by 103%, with 2010 exports sent to Mexico as refined Pb; to Canada, China, and Japan in spent Pb-acid batteries; and, to the Republic of Korea as Pb in concentrate (USGS, 2012). Primary Pb processing decreased by 67%, while Pb mining and imports fluctuated over 1991-2010 without a clear increasing or decreasing trend. In 2008, 1.28 million tons of Pb were introduced to the market by primary and secondary processing combined. Nine hundred sixty-four (964) tons of Pb were emitted to the ambient air. Hence, 99.9% of Pb produced in 2008 remained in products or was emitted directly to soil or water following disposal.



Note: Exports are shown by negative numbers to illustrate that the Pb was leaving the U.S. Data were aggregated into five-year totals to stabilize the data shown.

Source: U.S. Geological Survey (2012, 2006, 2001, 1996)

Figure 2-6 Five-year totals for Pb mining, primary and secondary production, imports, and exports, 1991-2010.

2.2.2.1 Pb Emissions from Piston-engine Aircraft Operating on Leaded-Aviation Gasoline and Other Non-road Sources

The largest source of Pb in the NEI, in terms of total emissions nationally, is emissions from piston-engine aircraft operating on leaded aviation gasoline (U.S. EPA, 2011a). As outlined in Table 2-1, there are several forms of Pb emitted from engines operating on leaded fuel. Dynamometer testing has indicated that Pb emissions from piston engines operating on leaded fuel can occur in the particulate and gaseous forms. For example, Gidney et al. (2010) performed dynamometer testing on automobiles operating on standard gasoline and on gasoline with low levels of organometallic additives. Tetraethyl Pb was included since it is still used in piston-engine aircraft fuel. Gidney et al. (2010) point out that, where tetraethyl Pb is used as an additive in piston-engine aircraft fuel, the fuel also contains ethylene dibromide, which reacts with Pb to form Pb bromide and Pb oxybromides. Pb bromides and Pb oxybromides are more volatile than elemental Pb at combustion temperatures and are therefore exhausted from the engine. After being exhausted, the brominated Pb compounds cool to ambient temperatures and condense to form sub-micron solid particles. In contrast, emissions of organic Pb would remain largely in the vapor phase at ambient temperatures. Studies of Pb emissions within enclosed microenvironments where automobiles were the dominant Pb source cited within the 1986 Pb AQCD (U.S. EPA, 1986a), reported that organic Pb vapors contributed less than 20% of total vehicular Pb emissions. A more recent study supports this (Shotyk et al., 2002). The 20% estimate of organic Pb emissions from the previous studies of on-road Pb emissions may potentially provide an upper bound for organic Pb emissions from current piston-engine aircraft.

Pb emission rates from piston aircraft vary with fuel consumption rates, which depend on the engine/airframe combination and the mode of operation of the aircraft. The ASTM specification for the maximum Pb content in “100 Low Lead,” the most commonly used leaded piston-engine aircraft fuel, is 2.12 g of elemental Pb/gallon (ASTM, 2007). Fuel consumption rates can be obtained for some engine/aircraft combinations by running FAA’s Emissions and Dispersion Modeling System (FAA, 2011). Fuel consumption for piston-engine aircraft operating at one airport in the U.S. was estimated to range from 1.6 grams per second (g/second) of fuel during taxi-out, to 15.3 g/second of fuel during run-up preflight check for single-engine aircraft; and 5.1 g/second during taxi and 50 g/second during preflight run-up check for twin-engine aircraft (Carr et al., 2011). Fuel consumption rates for aircraft listed in FAA’s Emissions and Dispersion Modeling System were used to develop the Pb emissions inventory for piston aircraft that are discussed in Section 2.2.1. EPA estimates that on average, 7.34 g of Pb is emitted during a landing and take-off cycle conducted by piston-engine aircraft (ERG, 2011).

2.2.2.2 Emissions from Metals Processing and Mining

High Pb emissions were observed in the 2008 NEI (U.S. EPA, 2011a) in Herculaneum, MO, where the Doe Run Pb smelter is operated. Although it is set to cease smelting operations in 2014 (DRRC, 2010), it is of interest to consider studies of primary smelter emissions in the context of the data analyzed in this ISA. Batonneau et al. (2004) and Sobanska et al. (1999) found that the Pb content in PM emitted from a primary Pb smelter was 56.6% by weight, and the Pb content in PM from a Pb/Zn smelter was 19.0% by weight. Choel et al. (2006) confirmed that Pb was strongly associated with sulfur in Pb-Zn smelter emission PM, and that Pb sulfates and Pb oxy-sulfates were the most abundant species, with important contributions from Pb oxides. Pb concentrations 1,800 meters downwind of the smelter (0.625-0.880 $\mu g/m^3$) were roughly thirty-five times higher than a monitor 1,800 meters upwind (0.017-0.026 $\mu g/m^3$).

Fugitive emissions (i.e., unaccounted ambient air Pb emissions) from secondary Pb processing (e.g., Pb recovery from batteries) can be substantial over the course of a year, but they are difficult to estimate. Thurston et al. (2011) performed source apportionment of $PM_{2.5}$ found that Pb-$PM_{2.5}$ concentrations from the Chemical Speciation Network (CSN) were associated with the metals industry along with Zn-$PM_{2.5}$. Goyal et al. (2005) estimated fugitive emissions using concentration data obtained from samplers sited in close vicinity of secondary Pb processing facilities and meteorological data from nearby weather monitoring stations. Regression modeling and Bayesian hierarchical modeling were both used to estimate fugitive and stack emissions from secondary Pb processing facilities in Florida, Texas, and New York. Depending on the model used, median fugitive emissions were estimated to be 1.0×10^{-6} to 4.4×10^{-5} g Pb/m^2 per second at the Florida site, 9.4×10^{-7} to 2.0×10^{-6} g/m^2 per second for the Texas site, and 8.8×10^{-7} to 1.1×10^{-6} g/m^2 per second at the New York site. Median stack emissions estimates varied widely among the models, with the Florida site median ranging from 1.4×10^{-6} to 1.4×10^{-1} g Pb/second, the Texas site median ranging from 8.4×10^{-2} to 8.6×10^{-2} g/second, and the New York site ranging from 8.4×10^{-3} to 1.0×10^{-2} g/second. Additionally, the Bayesian hierarchical model was used to estimate fugitive Pb emissions from secondary Pb processing facilities nationwide using concentration data as prior information. Nationwide median fugitive emissions from secondary Pb processing facilities were estimated to be 9.4×10^{-7} to 3.3×10^{-6} g/m^2 per second. Recently, speciation of emissions from a battery recycling facility indicated that PbS was most abundant, followed by Pb sulfates ($PbSO_4$ and $PbSO_4$-PbO), PbO and Pb^0 (Uzu et al., 2009).

In addition to secondary Pb smelting, Pb emissions occur from processing of other metals. For example, a recent study examined Pb emissions from a sintering plant, a

major component of the steel making process in southern France (Sammut et al., 2010). Cerussite, a Pb carbonate ($PbCO_3$-$2H_2O$), was observed to be the most abundant species and contributed 20 g Pb/kg measured PM. In another example, Reinard et al. (2007) used a real-time single particle mass spectrometer to characterize the composition of PM_1 collected in Wilmington, Delaware in 2005 and 2006. Strong Pb-Zn-K-Na associations were observed within 13% of PM samples. Comparison with stack emissions revealed that a nearby steel manufacturing facility was an important source of Pb. Ambient PM classes containing only a subset of such elements, e.g., Zn only, Pb-K only were non-specific and so could not be mapped to individual sources. Ogulei et al. (2006) observed that 6% of Pb in $PM_{2.5}$, along with some O_3, Cu, and Fe, was attributed to steel processing in Baltimore, MD. Murphy et al. (2007) conducted a detailed study of the distribution of Pb in single atmospheric particles during the fifth Cloud and Aerosol Characterization Experiment in the Free Troposphere campaign at the Jungfraujoch High Altitude Research Station in Switzerland and found that the predominant type of urban Pb-bearing aerosols contained Pb together with K and Zn. The mode of the size distribution for this type was around 200 nm.

Waste from current or defunct mines has been shown to present an additional fugitive source of Pb. For example, distribution of Pb along a haul road connecting an active mine to a port has been documented in Alaska (see Section 2.6.6). Additionally, Zheng and Li (2009) applied source apportionment in three northeastern Oklahoma towns to identify the influence of "chat," or waste piles from formerly operational Pb-Zn mines, on $PM_{10\text{-}2.5}$ and $PM_{2.5}$. They estimated that mine waste was responsible for 88% of Pb in $PM_{10\text{-}2.5}$ samples and 40% of Pb in $PM_{2.5}$ samples.

2.2.2.3 Fossil Fuel Combustion

Murphy et al. (2007) found that the volatility of Pb and its compounds such as PbO may result in its presence at high concentration in the submicron fraction of PM emitted from coal emissions. $PbSO_4$, also derived from coal combustion, has low water solubility (Barrett et al., 2010). $PbSO_4$ was estimated to comprise 37% of Pb in PM_{10} from a 2002-2003 study of PM in Shanghai, China (Tan et al., 2006) and 0.6% of total PM_{10} from a study of PM in Taiyuan City, China (Xie et al., 2009). Murphy et al. (2007) presented an estimated U.S. mass budget for Pb emitted from consumption of select fuels and crude oil. Fuel consumption estimates for 2005 were employed (Freme, 2004). Based on an annual consumption of 1.0×10^9 metric tons coal with an average Pb concentration of 20 mg/kg (range: 5 to 35 mg/kg) and using an emission factor (airborne fraction) of approximately 0.01, coal contributed approximately 200 metric tons Pb/year to the atmosphere. At the time of the Murphy et al. (2007) study, there were no emission factors

available to estimate airborne Pb emissions for crude oil or residual oil, but these represent potentially large sources (with total Pb in these sources estimated by Murphy et al. (2007) to be as much as 100-500 metric tons/year and 25-700 metric tons/year, respectively). These calculations imply that there is substantial uncertainty in estimates of Pb emissions resulting from fuel combustion. It is important to note that Murphy et al. (2007) state that the crude oil estimates are based on a limited number of samples and that there was uncertainty in the estimates of Pb content in residual oil. Furthermore, Murphy et al. (2007) was based on data ranging back in time from 1972 to 2005. Therefore, the Murphy et al. (2007) findings do not necessarily conflict with reported ambient air Pb emissions from the NEI. As part of recent rulemaking, EPA has developed a draft Pb emission factor of $1.3x10^{-5}$ lb/mmBtu for boilers larger than 25 MW that use #2 or #6 fuel oil (U.S. EPA, 2011b).The amounts of Pb emitted from these U.S. sources, however, are several orders magnitude smaller than those estimated to arise from coal combustion in China.

Coal combustion is considered to be a major source of Pb in the atmosphere now that leaded gasoline has been phased out for use in on-road vehicles (Diaz-Somoano et al., 2009). Global Pb estimates are considered here to inform understanding of U.S. Pb emissions from coal combustion. McConnell and Edwards (2008) examined correlations of Pb with BC, Cd, Ce, sea salt Na, non-sea salt S, and Tl in a Greenland ice core and observed high correlations for BC, Cd, non-sea salt S and Tl during the period 1860-1940, when coal combustion was the predominant energy source. With the exceptions of non-sea salt S and Tl, the high correlations were not maintained into the years 1940-2003, when oil combustion was the most prevalent energy source. This suggests common industrial sources of PbS or $PbSO_4$. Rauch and Pacyna (2009) constructed global metal cycles using anthropogenic data from 2000. They confirmed that the largest anthropogenic airborne Pb emissions arise from fossil fuel combustion, and they quantified Pb emissions at 85,000 tons/year worldwide. Globally, Pb emissions from stationary sources have been increasing and the north-south gradient in aerosol Pb concentrations over the Atlantic Ocean has disappeared as a result of industrialization of the southern hemisphere (Witt et al., 2006; Pacyna and Pacyna, 2001). The Pb isotope ratio values (mainly $^{206}Pb/^{207}Pb$) for coal from around the world have been compared with those for atmospheric aerosols. In most parts of the world, there has been a difference between the signature for aerosols and that for coal, where the atmospheric $^{206}Pb/^{207}Pb$ ratio values are lower, indicative of additional contributions from other sources. Zhang et al. (2009a) used single particle aerosol mass spectrometry (ATOFMS) to find that PM containing Pb along with OC and/or EC was attributed to coal combustion processes in Shanghai, China; this accounted for roughly 45% of Pb-bearing PM.

Seasonal effects of the contributions of Pb emissions from coal combustion have been observed. For example, in Tianjin, northern China, the winter heating period starts in November, and the contribution from coal combustion to the Pb aerosol becomes high during the winter. This leads to both a high Pb content and a high $^{206}Pb/^{207}Pb$ ratio. Coal consumption and Pb-bearing PM concentrations declined during the summer months, and Pb from other sources, mainly vehicle exhaust emissions, became relatively more pronounced (Wang et al., 2006c). This seasonal relationship contrasts with observations for the U.S. when power stations are more active in summer months (EIA, 2012). The increased energy use in summer periods in the U.S. may be attributable to increased requirements for air-conditioning.

2.2.2.4 Waste Incineration

Waste incineration studies suggest that the Pb content varies by industrial or municipal waste stream. For example, Ogulei et al. (2006) performed positive matrix factorization of $PM_{2.5}$ and gaseous copollutants for Baltimore, MD and observed that 63% of Pb in $PM_{2.5}$ was attributed to waste incineration during the six day study duration. Other prevalent compounds associated with incineration included NO_3^-, EC, Cd, Cu, Fe, Mn, Se, Zn, O_3, and NO_2 (note that Cl was not observed in this study). Likewise, Song et al. (2001) used PMF to deduce sources of $PM_{2.5}$ measured at Washington, D.C., Brigantine, NJ, and Underhill, VT during the years 1988-1999. They observed a waste incineration source loaded with OC, EC, Pb, and Zn at all three sites. A study by Moffet et al. (2008b) found that Pb-Zn-Cl-containing particles in $PM_{2.5}$ samples collected from an industrial area in Mexico City represented as much as 73% of fine PM. These were mainly in the submicron size range and were typically mixed with elemental carbon (EC), suggesting a combustion source. Zhang et al. (2009a) also observed high correlation between Pb and Cl associated with waste incineration in Shanghai, China. Several Pb isotope studies have also been used to distinguish contributions to incineration from industrial sources. Isotope analysis is discussed in more detail in Section 2.4.1.5. Novak et al. (2008) evaluated changes in the amounts and sources of Pb emissions in the U.K. and Czech Republic during the 19th and 20th centuries and found uncertainty in the amount and the isotope composition of Pb emanating from incineration plants. The isotopic signature of Pb recycled into the atmosphere by incineration of various industrial wastes could have shifted from relatively high $^{206}Pb/^{207}Pb$ ratios consistent with local Variscan ores to lower values reflecting imported Precambrian ores. However, other environmental studies concerning incineration have given highly consistent values for the Pb isotope ratio for European incineration sources. For example, Cloquet et al. (2006) showed that the Pb isotopic composition of urban waste incineration flue gases in northeastern France was

~1.16. De la Cruz et al. (2009) reported that waste incineration was an important source of Pb and showed that the $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{207}$ Pb ratios for waste incineration Pb emitted in European countries were 1.14-1.16 and 2.43 respectively (de la Cruz et al., 2009).

2.2.2.5 Wood Burning and Wildfires

Another potentially uncontrollable source is Pb deposited historically in forests and remobilized during forest fires. Section 3.1.3.1 describes residential Pb-PM concentrations related to in-home burning of wood contaminated with Pb of ambient origin, while this section describes ambient air measurements of Pb attributed to wood burning. The 2006 Pb AQCD (U.S. EPA, 2006b) presented data by Nriagu (1989) estimating that 1,900 metric tons of Pb were emitted globally each year from wildfires. Wildfire Pb emissions were not included in the NEI. Murphy et al. (2007) observed that a fraction of particles contained small quantities of Pb on biomass particles measured using ATOFMS to sample directly from forest fire plumes in northwest Canada and eastern Alaska in July, 2004; these particles also typically contained SO_4^{-2}. Several studies illustrate moderate-to-long range transport of biomass burning plumes containing Pb. Using positive matrix factorization, Ogulei et al. (2006) estimated that 20% of Pb in $PM_{2.5}$ measured in Baltimore, MD was attributed to a July, 2002 episode of wildfires in Quebec, Canada in his 6-day study. Other components strongly associated with the Quebec wildfires included NO_3^-, OC, EC, Cd, Mn, Zn, O_3, and CO. Qureshi et al. (2006) also observed a spike up to 42 ng/m^3 in Pb-$PM_{2.5}$ concentration in Queens, NY coinciding with the Quebec wildfires; for comparison, the authors provide the 3-month average from July to September of 5.1 ng/m^3 for Pb-$PM_{2.5}$ in Queens. Similarly, Anttilla et al. (2008) measured PM_{10} in Virolahti, Finland during a wildfire in Russia and observed average Pb-PM_{10} concentrations during the forest fire episodes to be 1.7-3.0 times higher than the reference concentration of 3.5 ng/m^3. Hsu et al. (2009c) observed Pb concentrations in Taiwan attributed to biomass burning in Northeastern China; Pb was highly correlated with K attributed to biomass burning during these episodes. Odigie and Flegal (2011) studied remobilization of Pb during the 2009 wildfires in Santa Barbara, CA. Pb concentrations in ash samples obtained after the wildfire ranged from 4.3 to 51 mg/kg. Isotopic analysis of the ash suggested that the remobilized Pb was initially emitted by a mix of contemporary and previous industrial sources and historic combustion of leaded gasoline. Grouped with “miscellaneous” Pb emissions, fires from agricultural field burning and prescribed fires accounted for 2.4 tons of U.S. Pb emissions in 2008 (U.S. EPA, 2011a). Polissar et al. (1998) used positive matrix factorization to apportion $PM_{2.5}$ and found small Pb signals attributed to the forest fire factor at two of

six Alaskan sites where a forest fire factor was detected; the forest fire factor was dominated by a combination of BC, H^+, and K.

Wood combustion emissions from commercial boilers present an additional potential Pb source. For example, Pb concentrations in 132 samples of wood pellets and 23 samples of wood chips from the northeastern US were presented by Chandrasekaran et al. (2012). Some of the commercially available pellets contained waste wood that was thought to include Pb-painted material. $PM_{2.5}$ emissions from the boilers were enriched with Pb as well as Cd, Ti, Rb, and Zn (Chandrasekaran et al., 2011). The concentration of Pb in $PM_{2.5}$ was 96% and 87% of the Pb concentration in pre-combustion wood pellet fuels of 0.014 kg/kg and 0.027 kg/kg, respectively.

Several studies have explored the chemical properties of biomass emissions. Obernberger et al. (2006) simulated biomass combustion in a laboratory setting to assess emissions. They reported pre-combustion mean Pb content in wood, bark, and logging residues to range from 2-5 mg/kg dry basis. They reported volatilization and subsequent condensation of Pb emissions from combustion. Van Lith et al. (2008; 2006) studied the inorganic element content of wood chips and particle board and the release of inorganic elements during combustion of those materials in laboratory experiments. They measured a Pb content of 16 mg/kg dry basis in particle board and of 0.44 mg/kg dry basis in spruce wood chips. Using three different types of combustion for different materials, they found that up to 10% of Pb was released at a combustion temperature of 500 °C and up to 85% was released at a temperature of 850 °C. At temperatures greater than 650 °C, PbO gas was released under oxidizing conditions; under reducing conditions, Pb gas, PbCl gas, and PbS gases were released at temperatures above 500 °C. Jimenez et al. (2008) performed laboratory experiments of olive tree combustion and concluded that Pb vaporizes upon combustion and then condenses between 900 °C and 560 °C. Jimenez et al. (2008) also observed that Pb concentration in PM changes with oxygen content and temperature, with concentrations converging toward 2,000 mg/kg for increasing percent available oxygen and increasing temperature.

Pb deposition on trees has been documented in Acadia National Park in Maine with mean foliar concentrations ranging from <0.5 to 3.1 mg/kg (Wiersma et al., 2007). Tree ring core samples obtained in the Czech Republic illustrate that the amount of Pb deposited on trees from coal and leaded gasoline combustion sources tended to increase over the depth of the core, with maximum concentrations corresponding to time periods of 1969-1972, 1957-1960, and 1963-1966 in three samples (Zuna et al., 2011).

2.2.2.6 Roadway-Related Sources

Contemporary Emissions from Vehicle Parts

Contemporary Pb emissions from motor vehicles may occur because several vehicle parts still contain Pb. Wheel weights, used to balance tires, are clipped to the rims of tire wheels in order to balance the tires, and may become loose and fall off. Pb wheel weights have been banned in several states including Washington, Maine, and Vermont with legislation considered in Iowa, California, and Maryland. However, Pb wheel weights are a source in most states for the period of time covered in this assessment. Ambient air Pb concentrations near heavily trafficked areas may be related to use of Pb-based wheel weights that are prone to dislodgement. Root (2000) and Aucott and Caldarelli (2012) estimated that 7.5 kg/km per year are deposited and that, among deposited weights, 2.7-5% of the mass is lost from the roadway daily. Aucott and Caldarelli (2012) extrapolated their results for Mercer County, NJ to the U.S. to estimate that 480 tons of Pb are deposited to roadways each year. On pavement they may be ground into dust by the pounding forces of traffic (Root, 2000). For example, Aucott and Caldarelli (2012) estimated that 13.8 ± 5.0% of the deposited mass of wheel weights are dispersed each year through abrasion and grinding by traffic. Schauer et al. (2006) measured Pb emissions in two traffic tunnels and found that the Pb-$PM_{2.5}$ concentration did not exceed 17% of the Pb-PM_{10} concentration in any of the runs. Schauer et al. (2006) suggested that enrichment in the coarse fraction may have been related to wheel weights. Additionally, Schauer et al. (2006) measured PM_{10} and $PM_{2.5}$ composition from brake dust and found concentrations that were low but statistically significantly greater than zero for Pb in PM_{10} (0.02 ± 0.01 mg/g) and Pb in $PM_{2.5}$ (0.01 ± 0.00 mg/g) for semi-metallic brake pads and for Pb in PM_{10} (0.01 ± 0.00 mg/g) for low-metallic brake pads. Song and Gao (2011) speciated coarse and fine PM samples obtained next to the New Jersey Turnpike in winter and summer of 2007-2008. Using principal component analysis, they found that Pb was prevalent in the factor including automobile exhaust and brake wear. Pb was observed to have a similar size distribution as Zn in the winter and Zn and Cd in the summer, with higher concentrations in the fine fraction at a mode of 0.18-0.32 µm. Fauser (1999) observed that 92% of particles generated by tire abrasion have aerodynamic diameter smaller than 1 µm. Additionally, Hjortenkrans et al. (2007) used material metal concentrations, traffic volume, emissions factors, and sales data to estimate the quantity of Pb emitted from brake wear and tires in Stockholm, Sweden in 2005. They observed that 24 kg (0.026 ton) of Pb were emitted from brake wear each year, compared with 2.6 kg (0.0029 ton) of Pb from tire tread wear; an estimated 549 kg (0.61 ton) was estimated to have been emitted from brake wear in 1998. McKenzie et al. (2009) determined the composition of various vehicle components including tires and

brakes and found that tires were a possible source of Pb in stormwater, but no identification of Pb-containing PM in stormwater was carried out. However, PM from tire abrasion is usually found in coarser size ranges (Chon et al., 2010), while those in the submicron range are more typically associated with combustion and incineration sources.

Road Paint

Some paints, frequently used for road markings and near-road structures, have contained large quantities of Pb. Ozaki et al. (2004) analyzed paints used in urban and forested areas of Japan to delineate traffic lanes on roads and measured high concentrations of Pb (12 mg/g) and Cr (820 mg/g) in yellow paint, suggestive of $PbCrO_4$. High concentrations of Pb (5.3 mg/g) and Cr (6.7 mg/g) were measured in red paint, also suggesting presence of $PbCrO_4$. Gray paint and anticorrosive used to coat guardrails was also measured to have high Pb concentrations of 451 mg/g.

Street dust particles containing Pb, Cr, or both have been associated with $PbCrO_4$ in street paints. Adachi and Tainosho (2004) used scanning electron microscopy to analyze road dust composition and found particles with high Pb and Cr content, indicative of $PbCrO_4$ in yellow paint. Murakami et al. (2007) estimated enrichment of road dust by paint particles. They measured 46% of the road dust particles to contain more than 0.2% Pb, Cr, or both. Pb and Cr loading (mass deposited per area of surface) was also observed for all road dust size fractions measured (ranging from < 43 μm to > 841 μm) (Lau and Stenstrom, 2005).

Unleaded Fuel

Unleaded fuel contains Pb as an impurity within crude oil (Pacyna et al., 2007). Schauer et al. (2006) measured Pb in $PM_{2.5}$ from tailpipe emissions and observed quantities in on-road gasoline emissions that were statistically significantly different from zero (83.5 ± 12.80 mg/kg), whereas emissions of Pb from diesel engines were not statistically significantly different from zero. Hu et al. (2009a) investigated the heavy metal content of diesel fuel and lubricating oil. They found <1-3 mg/kg Pb in samples of lubricating oil. Hu et al. (2009a) also measured the size distribution of Pb emissions during dynamometer testing of heavy duty diesel vehicles with different driving patterns and control technologies. An urban dynamometer driving schedule (UDDS) designed to mimic urban stop-go driving conditions, was simulated in two cases to produce 80 and 241 ng Pb/km driven, depending on the control technology used. Respectively, 54% and 33% of those emissions were smaller than 0.25 μm in mass median aerodynamic diameter (MMAD). Song et al. (2001) and Polissar et al. (2001) both used positive matrix

factorization to decompose $PM_{2.5}$ samples obtained from an Underhill, VT site [Song et al. (2001) used data from 1988-1999 and Polissar et al. (2001) used data from 1988-1995] and observed a "Canadian Mn" factor loaded with Mn and Pb. Methylcyclopentadienyl manganese tricarbonyl had been used in Canada as an antiknock agent to replace tetraethyl and tetramethyl Pb additives, but it is not clear if the co-occurrence of Mn with Pb denotes that the phase-out had not been completed during part of the study period (Canadian phase-out completed in 1993).

2.2.2.7 Deposited Pb

Soil Pb can serve as a reservoir for deposited Pb. The following subsections describe studies of previously deposited Pb that originated from industrial activities, historical use of leaded on-road gasoline, and urban sources such as paint and building materials. The 2006 Pb AQCD (U.S. EPA, 2006b) cited an estimate by Harris and Davidson (2005) that more than 90% of airborne Pb emissions in the South Coast Basin of California were from soil resuspension. This value was obtained by constructing mass balances rather than from direct measurements of Pb along roads, and hence it is an estimate. Currently, measured data are not available with sufficient spatial resolution to discern the specific contribution of soil Pb resuspension to air Pb concentration, but resuspended soil Pb cannot be eliminated as a potential source of airborne Pb. Section 2.5.1.2 includes recent information on ambient air concentrations of Pb-TSP, sampled at 6 meters above ground level (AGL), at a distance of 500 meters from the heavily trafficked Interstate I-405, and 10 meters from a busy arterial road in Los Angeles. From this monitor, average concentrations were not substantially higher than the local urban background concentration (Sabin et al., 2006b). Insufficient data are available to ascertain if the near road Pb-TSP concentrations would be higher at lower monitor heights. The 2006 Pb AQCD (U.S. EPA, 2006b) also noted a smaller estimate of 40% for the Southern California Air Basin (Lankey et al., 1998).

In a recent paper, Laidlaw and Filipelli (2008) analyzed Interagency Monitoring of Protected Visual Environments (IMPROVE) data to explore conditions under which $PM_{2.5}$ particles estimated to be of crustal origins that may contain Pb may become airborne. They observed a seasonal pattern in the concentration of $PM_{2.5}$ of crustal origins in the atmosphere, and they also found that at one IMPROVE site in central Illinois, 83% of the variability in concentrations of crustal $PM_{2.5}$ was predicted by variability in meteorology and soil moisture content. The authors concluded that seasonality and climate parameters could not be eliminated in relation to ambient air Pb concentrations. Such mechanisms are described in more detail in Section 2.3. As described in Section 2.2.2.6 and Section 2.6.1, there are many contemporary

contributions of Pb to soil in urban areas, and studies summarized here have not quantitatively differentiated the contributions of these various sources to Pb concentrations in urban areas.

Pb from Industrial Activities

Several studies have indicated elevated levels of Pb are found in soil exposed to industrial emissions, including brownfield sites (Dermont et al., 2010; Verstraete and Van Meirvenne, 2008; Jennings and Ma, 2007; van Herwijnen et al., 2007; Deng and Jennings, 2006). Pb in industrial soils is described in Section 2.6.1. Recent Pb speciation results also indicate a contribution from resuspended soils in areas with previous major emission sources, but without current major sources. Data from airborne PM in the vicinity of an inactive smelter in El Paso, TX were described as consistent with Pb-humate as the major form of Pb in airborne PM, which the authors suggest relates to soil resuspension since the local near-surface soils appeared to have had high humic content (Pingitore et al., 2009).

Pb from Paint and Building Materials

Exterior structures painted with Pb-based paint have long been known to be a source of Pb in outdoor dust or grit (U.S. EPA, 2006b). Recent studies support earlier findings. Mielke and Gonzales (2008) sampled exterior paint chips from paint applied prior to 1992 on 25 homes in New Orleans, LA, and they found elevated Pb levels in 24 of the 25 tested exterior paints. Weiss et al. (2006) studied the distribution of Pb concentration in roadway grit in the vicinity of steel structures in New York City and contrasted those data with roadway grit concentration data where no steel structure was nearby. In each case, the difference was significant ($p < 0.006$ at one site and $p < 0.0001$ at 4 other sites), with median Pb concentrations in the grit under the steel structures (median: 1,480 mg/kg) collectively being 4.4 times higher than median Pb concentrations in the roadway grit not near a structure (median: 340 mg/kg).

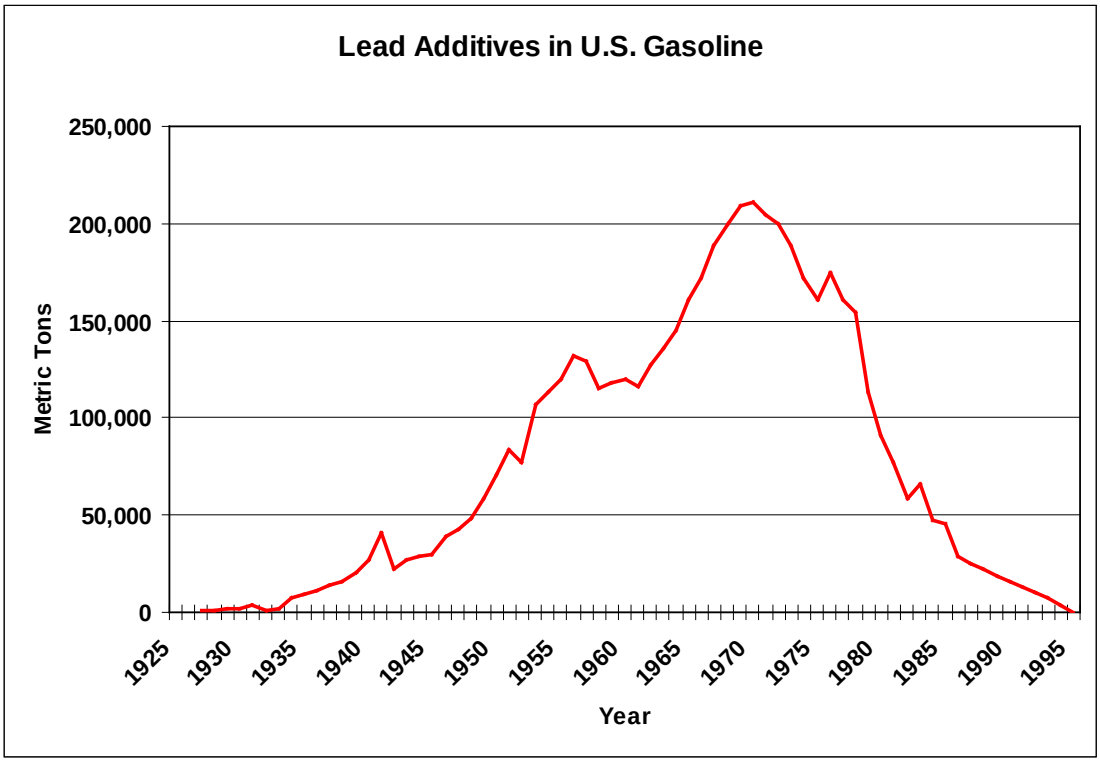
The studies described above considered paint as a source of Pb in outdoor dust through gradual abrasion of the painted surfaces. However, atmospheric conditions may break down polymers in aging paint, causing previously bound Pb-based pigments to be released from the surface more readily. Edwards et al. (2009) performed experiments to simulate one week of exposure of Pb-based paints to highly elevated levels of O_3 (11.3 ± 0.8 mg/kg or 150 times the level of the 8-hour NAAQS) and NO_2 (11.6 ± 0.9 mg/kg, or 220 times the level of the annual NAAQS). Following NO_2 exposure, the Pb in wipe samples increased by a median of 260% ($p < 0.001$), and

following O_3 exposure, the Pb in wipe samples increased by a median of 32% ($p = 0.004$).

Building demolition was listed as a source of Pb in urban dust in the 2006 Pb AQCD (U.S. EPA, 2006b). In a follow-up study to previous work cited therein, Farfel et al. (2005) observed that surface loadings of dust containing Pb increased by 200% in streets, by 138% in alleys, and by 26% in sidewalks immediately following demolition of an old building compared with surface loadings of dust containing Pb prior to demolition. One month later, Pb dust loadings were still elevated in alleys (18% higher than pre-demolition) and sidewalks (18% higher than pre-demolition), although they had decreased in streets by 29% compared with loadings prior to demolition. However, Farfel et al. (2005) did not provide detailed time series samples from before or after demolition to judge whether the observations made one month following demolition were within the normal conditions of the urban area. These results suggest that building demolition may be a short-term source of Pb in the environment, but it is unclear if demolition is related to long-term Pb persistence in the environment.

Pb from Historic Automobile Emissions

Historic Pb emissions, or Pb emitted from on-road vehicles prior to the ban on use of leaded automobile gasoline, deposited onto soil and in some areas may serve as a potential source of airborne Pb. The historical use of leaded on-road gasoline has been estimated from documents submitted by Ethyl Corporation to the U.S. Senate (1984) and a report by the U.S. Geological Survey (USGS, 2005); see Mielke et al. (2011c). These estimates are presented in Figure 2-7. The peak U.S. use of Pb additives occurred between 1968 and 1972 with an annual amount of over 200,000 metric tons. According to Ethyl Corporation, the 1970 use of Pb additives was 211,000 metric tons. By 1980, the annual use of Pb additives to on-road gasoline decreased to about 91,000 metric tons or a 57% reduction from its 1970 peak. From 1970 to 1990 there was a 92% decline in Pb additive use. In 1990, the annual U.S. use of Pb additives decreased to 16,000 metric tons, a further 82% decline in Pb additive use from 1980. The final U.S. ban on the use of Pb additives for highway use in on-road gasoline occurred in 1996. After that time, Pb additives were only allowed in nonroad applications, including piston-engine aircraft fuel, racing fuels, farm tractors, snowmobiles, and boats.



Note: Estimates were derived from the proceedings of the U.S. Senate hearings on the Airborne Pb Reduction Act of 1984, S. 2609 (1984) and the U.S. Geological Survey Pb end use statistics (USGS, 2005).

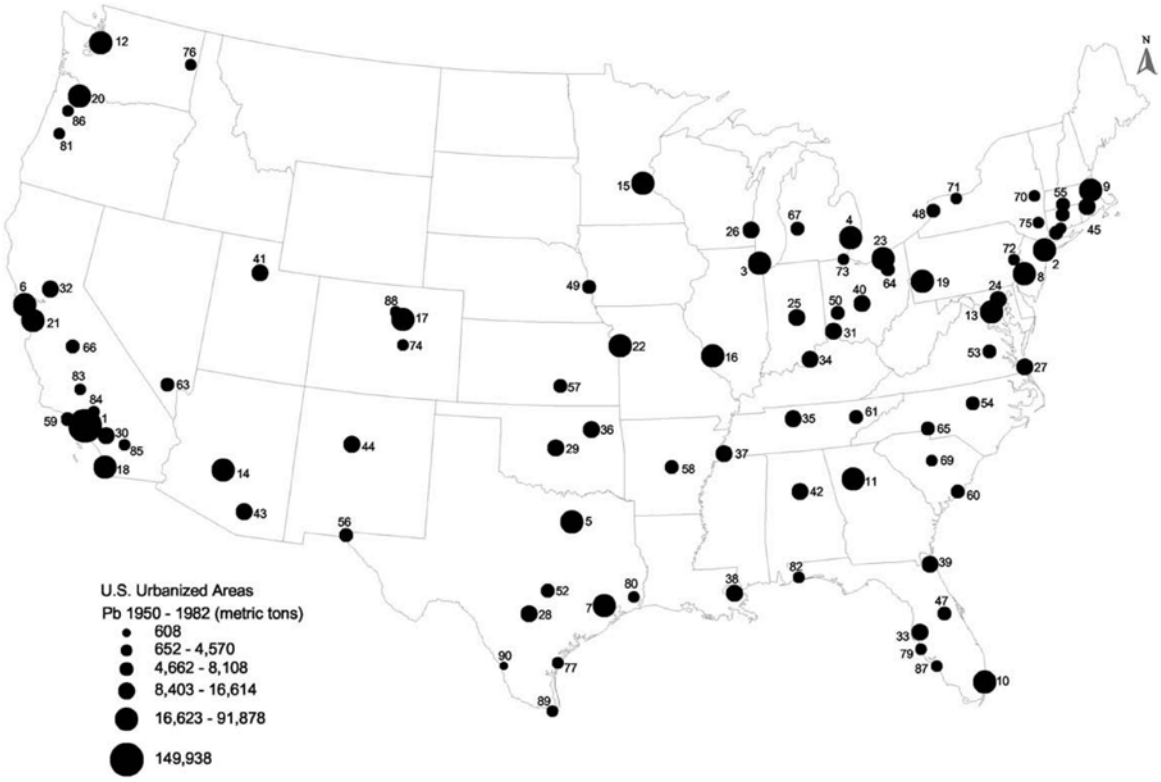
Source: Reprinted with permission of Pergamon Press, Mielke et al. (2011c).

Figure 2-7 Total U.S. Pb additives in on-road gasoline used in on-road vehicles, 1927-1995.

Pb emissions from on-road sources were estimated by the U.S. EPA (1986a), which indicated that 75% of Pb additives were emitted as exhaust, while the remainder were retained within the engine. The tonnages of relatively large >10 µm mass median aerodynamic diameter (MMAD) Pb-PM probably settled locally. EPA (1986a) indicated that 35% of the Pb-PM at that time were <0.25 µm in MMAD. In high traffic urbanized areas, soil Pb from historic emissions as well as contemporary sources, are elevated adjacent to roadways and decrease with distance away from roadways (Laidlaw and Filippelli, 2008).

The use of Pb additives resulted in a national scale of influence. For example, variously sized urbanized areas of the U.S. have different amounts of vehicle traffic associated with Pb (Mielke et al., 2010). Figure 2-8 illustrates the national scale of the estimated vehicle-derived Pb aerosol emissions. Note that the estimated 1950-1982 Pb aerosol emissions in the 90 cities below vary from 606 metric tons for Laredo, Texas, to nearly 150,000 metric tons for the Los Angeles-Long Beach-Santa Anna urbanized area. Although this figure might imply that the soil Pb concentration in these areas would be

proportional to the magnitude of historic on-road emissions in each city, it is recognized that the atmospheric dispersion of emissions, as well as the atmospheric deposition and subsequent distribution associated with surface runoff, will have varied substantially among the cities illustrated. The 2006 and prior CDs described the wealth of evidence documenting the long-range dispersion of Pb emitted from tail pipes beyond the U.S. and depositing in polar ice and seawater of both oceans (U.S. EPA, 2006b, 1986a, 1977). Additionally, the amount of soil turnover since 1982 may have varied substantially among the cities illustrated in Figure 2-8, depending on the amount of highway construction in those cities. As noted in Section 2.2.2.6, there have historically been, and are currently, many additional sources of Pb contributing to near-roadway soil Pb concentrations. Data are lacking that quantify the range of airborne Pb concentrations originating from historic Pb in resuspended soil particles, but data on airborne concentrations near roadways indicate measured air Pb concentrations (from all contributing sources) to be generally less than 0.02 $\mu g/m^3$ (Section 2.5.3.2).



Note: The numbers on the map are rankings of each urbanized area (UA). The size of each dot refers to the magnitude of motor vehicle gasoline-related emissions for each group of UAs.

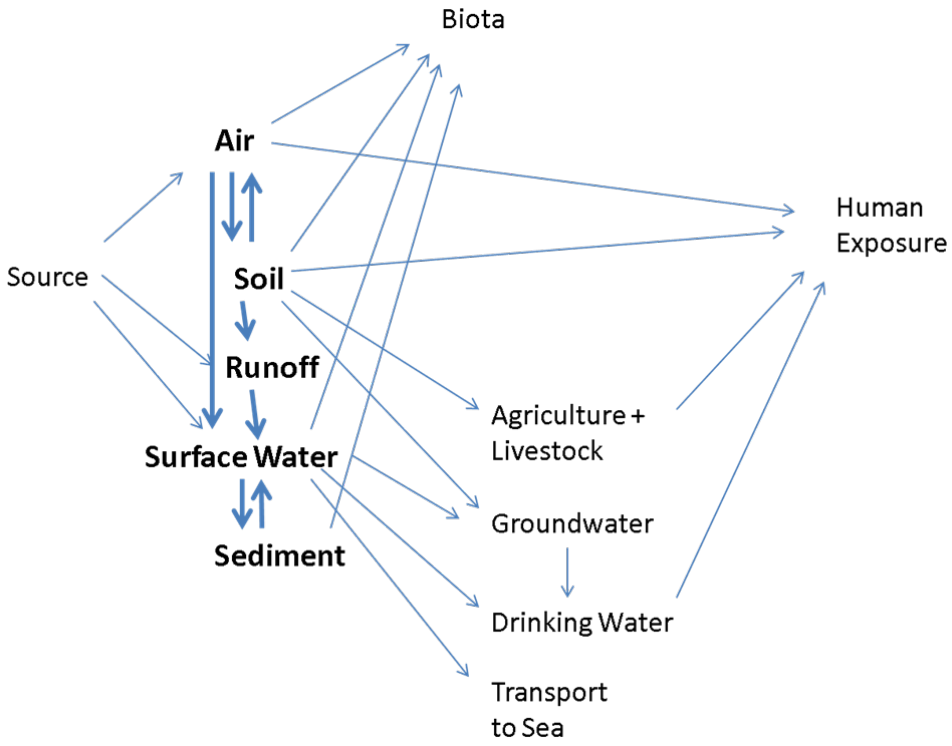
Source: Reprinted with permission of Pergamon Press, Mielke et al. (2011c)

Figure 2-8 Estimated Pb aerosol inputs from on-road gasoline into 90 U.S. urbanized areas (UAs), from 1950 through 1982.

2.3 Fate and Transport of Pb

There are multiple routes of exposure to Pb, including direct exposure to atmospheric Pb, exposure to Pb deposited in other media after atmospheric transport, and exposure to Pb in other media that does not originate from atmospheric deposition. As a result, an understanding of transport within and between media such as air, surface water, soil, and sediment is necessary for understanding direct and indirect impacts of atmospheric Pb as well as the contribution of atmospheric Pb to total Pb exposure through inhalation and ingestion pathways. Figure 2-9 describes relevant Pb transport pathways through environmental media discussed in this chapter and their relationship to key environmental and human exposure pathways for which some or all of the Pb is processed through the atmosphere. This discussion includes recent research on atmospheric transport of Pb, atmospheric deposition and resuspension of Pb, Pb transport in surface waters and

sediments, and Pb transport in soil. Facets of fate and transport relevant to the ecological effects of Pb are also summarized in Section 6.2.



Note: Media through which Pb is transported and deposited are shown in bold.

Figure 2-9 Fate of atmospheric Pb.

2.3.1 Air

The 2006 Pb AQCD (U.S. EPA, 2006b) concluded that Pb was primarily present in submicron aerosols, but that bimodal size distributions were frequently observed. Pb-PM in the fine fraction is transported long distances, found in remote areas, and can be modeled using Gaussian plume models and Lagrangian or Eulerian continental transport models as reported by several studies. Good agreement between measurements and these models have been reported. Historical records of atmospheric deposition to soil, sediments, peat, plants, snowpacks, and ice cores have provided valuable information on trends and characteristics of atmospheric Pb transport. Numerous studies using a variety of environmental media indicated a consistent pattern of Pb deposition peaking in the 1970s, followed by a more recent decline. These findings indicated that the elimination of leaded gasoline for motor vehicles and systematic reductions in emissions from other Pb

sources has not only led to lower atmospheric concentrations in areas impacted by vehicles (Section 2.5), but a pervasive pattern of decreasing atmospheric Pb deposition and decreasing concentrations in other environmental media even at great distances from sources.

2.3.1.1 Transport

Recent research on long range transport as well as transport of Pb in urban areas has advanced the understanding of Pb transport in the atmosphere. While the 2006 Pb AQCD described long range Pb transport as essentially a process of submicron PM transport (U.S. EPA, 2006b), much of the recent research on Pb transport has focused on interactions between anthropogenic and coarser geogenic PM that leads to incorporation of Pb into coarse PM as well as subsequent transformation on exposure to mineral components of coarse PM. Using scanning electron microscopy (SEM), Schleicher et al. (2010) observed interactions of anthropogenic soot and fly ash particles on the surfaces of coarse geogenic mineral particles in Beijing, China and concluded that toxic metals were often associated with TSP. Murphy et al. (2007) found that PM released from wild fires and transported over long distances scavenged and accumulated Pb and sulfate through coagulation with small Pb rich PM during transport and that Pb was associated with PM over a wide size range. Erel et al. (2006) also found that Pb enrichment factors calculated for PM_{10} from dust storms collected in Israel were much greater than those sampled at their north African source, suggesting that the dust samples had picked up pollutant Pb in transit between the Saharan desert and Israel. Marx et al. (2008) characterized dust samples collected from the surface of glaciers and in dust traps on the remote west coast of New Zealand's South Island and observed that most of the dust samples were enriched in metals, including Pb, compared with their source area sediments.

Pb accumulated on mineral dusts is also subject to atmospheric transformations. $PbSO_4$ is one of the main constituents of Pb-containing aerosols resulting from coal combustion (Gieré et al., 2006) and it has been shown to react with calcite, $CaCO_3$, a PM mineral component, to form $Pb_3(CO_3)_2(OH)_2$, $Pb(CO_3)$ and $Ca(SO_4)_2 \cdot H_2O$ on the surface of the calcite (Falgayrac et al., 2006). In laboratory experiments, (Ishizaka et al., 2009) also showed that $PbSO_4$ could be converted to $PbCO_3$ in the presence of water. Approximately 60-80% was converted after only 24 hours for test samples immersed in a water droplet. This compared with only 4% conversion for particles that had not been immersed. As a result of recent research, there is considerable evidence that appreciable amounts of Pb can accumulate on coarse PM during transport, and that the physical and

chemical characteristics of Pb can be altered by this process due to accompanying transformations.

2.3.1.2 Deposition

The 2006 Pb AQCD (U.S. EPA, 2006b) documented that soluble Pb was mostly removed by wet deposition, and most of the insoluble Pb was mostly removed by dry deposition. As a result, dry deposition was the major removal mechanism for Pb in coarse PM (which is mainly insoluble and settles faster than fine PM), and wet deposition was the most important removal mechanism for fine PM and Pb halides (which were more soluble). Numerous studies reported that Pb dry deposition velocities in the U.S. were mostly within a range of 0.05 to 1.0 cm/second and dry deposition fluxes ranging from 0.04 to 4 mg/m^2 per year. Precipitation concentrations ranged mostly from 0.5 to 60 μg/L, but with considerably lower concentrations in remote areas, and wet deposition fluxes in the U.S. ranged from 0.3 to 1.0 mg/m^2 per year. Wet deposition was linked to precipitation intensity, with slow even rainfalls usually depositing more Pb than intense rain showers. Rain concentrations decreased dramatically between the early 1980s and the 1990s, reflecting the overall decreasing trend in Pb emissions due to elimination of leaded motor vehicle gasoline. A summary of studies investigating total deposition including both wet and dry deposition indicated typical deposition fluxes of 2-3 mg/m^2 per year and dry to wet deposition ratios ranging from 0.25 to 2.5. Seasonal deposition patterns can be affected by both variations in local source emissions and vegetation cover, and as a result a consistent seasonal pattern across studies has not been observed, although there have been only a few investigations. The 2006 Pb AQCD (U.S. EPA, 2006b) concluded that resuspension by wind and traffic contributes to airborne Pb near sources.

Wet Deposition

The 2006 Pb AQCD (U.S. EPA, 2006b) documented that dry deposition was the major removal mechanism for Pb in coarse PM and wet deposition as the most important removal mechanism for fine PM. Which process is most important for atmospheric removal of metals by deposition is largely controlled by solubility in rain water. Metal solubility in natural waters is determined by a complex multicomponent equilibrium between metals and their soluble complexes and insoluble ionic solids formed with hydroxide, oxide, and carbonate ions. This equilibrium is strongly dependent on pH and ionic composition of the rain water. As pH increases, Pb solubility is reduced. Theodosi et al. (2010) found that solubility of Pb was near 100% when rain water pH was measured to be less than 4.5. As a consequence, it is possible that efforts to reduce acidity

of precipitation could also reduce wet deposition of Pb. Recent research confirms the general trend described in the 2006 Pb AQCD (U.S. EPA, 2006b) that Pb associated with fine PM is usually more soluble in rain water than Pb associated with coarse PM, leading to a relatively greater importance of wet deposition for fine Pb and of dry deposition for coarse Pb. Theodosi et al. (2010) concluded that larger particles were less soluble, because Pb solubility decreased with increasing dust loading. Likewise, Preciado and Li (2006) observed that solubility decreased with increasing particle size. Moreover, Theodosi et al. (2010) observed that 53% of wet deposition samples were comprised of particulate Pb, not soluble Pb. This finding suggests that wash-out can be equally important to wet deposition as solubility.

Although recent observations are consistent with previous findings, they also indicate considerable spatial and seasonal variability. Birmili et al. (2006) found that Pb solubility varied between the two main Pb-containing size fractions, <0.5 μm (~40%) and 1.5-3.0 μm (~10%), indicative of a different chemical speciation. However, the observation that the amount of soluble Pb was higher in their U.K. samples than in an analytically identical study carried out in Seville, Spain (Fernandez Espinosa et al., 2004), led them to conclude that Pb solubility in fine PM may vary on a regional basis (Birmili et al., 2006). For PM_{10} from Antarctica, 90 to 100% of the Pb was insoluble at the beginning of the summer season (November), but by the end of the summer (January), approximately 50% was soluble. Most of the Pb was from long range transport (Annibaldi et al., 2007). These studies illustrate the variable nature of atmospheric Pb solubility.

Dry Deposition

Recent research on dry deposition has focused on differences between urban or industrial sites and rural or less industrial areas. For locations outside of industrial areas, new measurements of Pb dry deposition fluxes are similar to those reported in the 2006 Pb AQCD (U.S. EPA, 2006b), but in industrialized urban areas, they are considerably greater than in nonindustrialized areas. Deposition is typically documented by measurements of Pb concentrations on surface material or by measurements of flux. For example, Hasselbach et al. (2005) documented Pb concentration in moss, and the spatial distribution of Pb concentration in moss, as evidence of Pb deposition from truck traffic between an Alaskan Zn-Pb mine and a port. Additionally, Maher et al. (2008) measured Pb deposition onto leaves near a road in Norwich, U.K. Their results are described in detail in Section 2.6.6.

Several studies presented measurements of dry deposition flux obtained by capturing deposited particles onto a sampling substrate. Hence, these measurements did not provide

information on net deposition following resuspension of deposited material. Resuspension processes and measurements thereof are described in Section 2.3.1.3. Yi et al. (2006) calculated dry deposition fluxes for trace elements including Pb in New York-New Jersey harbor and observed much greater dry deposition fluxes for this urban industrial site in Jersey City (mean: 50 μg/m^2·day) than for suburban New Brunswick (mean: 8 μg/m^2·day). Sabin and Schiff (2008) measured dry Pb deposition flux along a transect from Santa Barbara to San Diego, CA in 2006 and observed a range of 0.52-14 μg/m^2·d for the median values across the eight sites. The highest median Pb flux was observed at Los Angeles Harbor, which is downwind of a harbor with a mix of industrial (harbor-related) and urban activities (14 μg/m^2·day). The second highest median Pb flux was observed at San Diego Bay, a military port (3.3 μg/m^2·day). This is consistent with similar observations of dry deposition fluxes that were more than ten times greater in urban Chicago than in rural South Haven, Michigan (Paode et al., 1998). These results illustrate the strongly localized nature of atmospheric Pb deposition in source rich areas. In a study of Tokyo Bay, Sakata and Asakura (2008) reported an average dry deposition velocity of 1.06 cm/second, which is near the upper end of dry deposition velocities reported in the 2006 Pb AQCD (U.S. EPA, 2006b). They also reported that dry deposition fluxes were greater in industrially impacted urban areas, ranging from 12-17 mg/m^2 per year.

Recent results also confirmed the trend of decreasing overall deposition fluxes after removal of Pb from on-road gasoline, as described in the 2006 Pb AQCD (U.S. EPA, 2006b). Watmough and Dillon (2007) found that the bulk annual deposition of Pb in a central Ontario forested watershed during 2002-2003 was 0.49 mg/m^2 per year; this was lower than the value of 1.30-1.90 mg/m^2 per year for 1989-91 and represented a 75% decline in Pb deposition. It was consistent with the decline more generally observed for the Northeastern U.S. as a consequence of the restrictions to alkyl-Pb additives in on-road gasoline. From previously published work, and in agreement with the precipitation data described above, most of the decline in Ontario Pb deposition took place before the start of the Watmough and Dillon (2007) study.

Within-day variation in deposition fluxes was observed to be related to the urban boundary layer. Lim et al. (2006) observed higher deposition fluxes of Pb and other metals during the nighttime in Los Angeles, when inversions are frequent occurrences. Deposition mass was also greater for particles larger than 10 μm in the urban areas where measurements occurred. Larger particle deposition flux was greater during the day.

Several important observations can be highlighted from the few studies of atmospheric Pb deposition carried out in the past several years. Deposition fluxes have greatly declined since the removal of Pb additives from on-road gasoline. However, more recent

results in industrial areas indicate that local deposition fluxes there are much higher than under more typical conditions. In general, wet deposition appears to be more important for Pb in fine PM, which is relatively soluble; and dry deposition appears to be generally more important for Pb in coarse PM, which is relatively insoluble. However, the relative importance of wet and dry deposition is highly variable with respect to location and season, probably reflecting both variations in Pb speciation and variations in external factors such as pH and rain water composition. Although industrial Pb emissions are mainly associated with fine PM, and wet deposition is likely to be more important for this size range, a substantial amount of Pb is apparently removed near industrial sources.

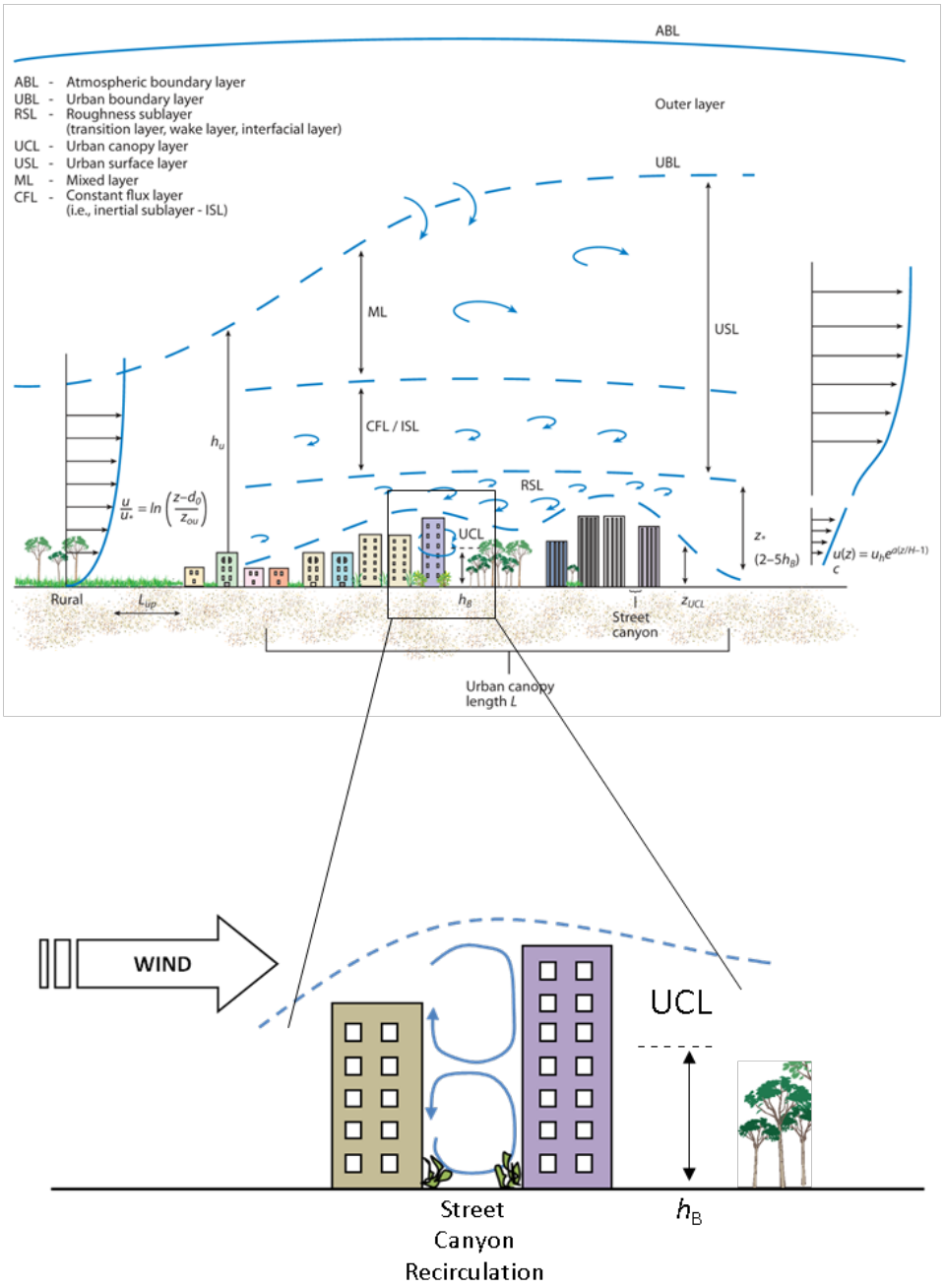
2.3.1.3 Resuspension of Pb from Surface Soil to Air after Deposition

The following information focuses on issues regarding the transport processes affecting resuspended soil Pb and dust Pb in urban environments. As described in Section 2.2.1, the greatest point source Pb emissions in the U.S. occur in locations near specific major facilities, such as secondary smelters, and other industrial operations involving large scale metal processing or fuel combustion. However, in the absence of such sources and in the vicinity of previous major sources, the 2006 Pb AQCD (U.S. EPA, 2006b) concluded that resuspension by mechanical stressors such as traffic, construction, and wind can be a source of airborne Pb above background levels near sources, with Pb accounting for between 0.002 to 0.3% of the mass of resuspended PM_{10}. Additionally, solubility of Pb increases its soil mobility, while adsorption to inorganic soil complexes decreases mobility (McLean and Bledsoe, 1992). These factors influence the site of Pb content within soil and hence its ability to become resuspended. Factors affecting Pb mobility in soil are discussed in more detail in Section 2.3.3.3. Reentrainment of deposited Pb complicates air related pathways of human (Section 3.1.1) and ecosystem exposure (Section 6.2.2).

Results from several studies have suggested minor contributions from resuspension to airborne Pb concentration from various sources, including city centers (Laidlaw and Filippelli, 2008), major freeways (Sabin et al., 2006b), and steel structures with abrading paint (Weiss et al., 2006). Recently, Laidlaw et al. (2012) modeled concurrent measurements of (log-transformed) air Pb-$PM_{2.5}$ as a function of (log-transformed) airborne soil measured in $PM_{2.5}$. They observed a statistically significant increase in air Pb of 0.84% with a 1% increase in airborne soil ($p < 0.01$). As noted in the 2006 Pb AQCD (U.S. EPA, 2006b), the contribution of resuspended soil and dust to the airborne burden may be significant from highly contaminated sites (e.g., active or abandoned industrial facilities and Superfund sites). In contrast, as summarized in

Section 2.5.3, Pb concentrations near roads in urban areas are one to two orders of magnitude below the current Pb NAAQS.

The urban environment can be considered quite different from natural landscapes because it has been highly modified by human activity, including above- and below-ground infrastructure, buildings, and pavement, and a high density of motorized transportation. These factors may influence the distribution and redistribution of Pb-bearing PM. As shown in Figure 2-10, urban turbulence occurs on several scales. Transport and dispersion of urban grit is subject to air movement within the urban canopy layer, where air movement is driven by air velocity within the urban boundary layer and urban topographical conditions such as building shape, building façade, and street canyon aspect ratio (Fernando, 2010). Within a street canyon, air circulates and tends to form counter-rotating eddies along the height of the canyon (Figure 2-10), which result in lower mean components of air movement, higher turbulence components, and higher shear stress within the canyon compared with open field conditions (Kastner-Klein and Rotach, 2004; Britter and Hanna, 2003). Recirculation around intersection corners and two-way traffic conditions can also enhance turbulence levels, while one-way traffic conditions increase air velocity along the street (Soulhac et al., 2009; Kastner-Klein et al., 2003; Kastner-Klein et al., 2001). Sedefian et al. (1981) measured the length scales of turbulent eddies resulting from passing 50 mph (22.2 meters/second) traffic on a test road and observed scales of 0.6-2.7 meters when winds were perpendicular to the test road and scales of 1.8-2.7 meters when winds were parallel to the road.



Note: Top: multiple scales within the atmospheric boundary layer. Bottom: illustration of airflow recirculation within a single street canyon located in the urban canopy layer.

Source: Adapted with permission of Annual Reviews, Fernando (2010)

Figure 2-10 Scales of turbulence within an urban environment.

Recent research on urban PM transport is highly relevant to Pb transport and dispersion because Pb is most prevalently particle-bound. Relevant results for Pb exposure in these areas include observations that PM concentration peaks dissipate more rapidly on wider streets than in narrow street canyons (Buonanno et al., 2011); concentrations are typically low next to a building because either less source material is available or less material penetrates the boundary layer of the building (Buonanno et al., 2011); and there are stronger inverse relationships between mean wind speed and PM concentration fluctuation intensities at middle sections of urban street blocks compared with intersections (Hahn et al., 2009). Patra et al. (2008) conducted experiments in London, U.K. in which a "tracer" grit (i.e., rock salt) was applied to a road and then the grit's dispersion by traffic was measured over time to simulate resuspension and transport of road dust. During the experiments, 0.039% of the tracer grit was measured to move down the road with each passing vehicle, 0.0050% was estimated to be swept across the road with each passing vehicle, and 0.031% was estimated to become airborne when a vehicle passed.

Harris and Davidson (2008) developed a model of resuspension of single particles initially at rest on a solid surface based on the balance of lift, drag, gravity, torque, and adhesion forces on the particle in addition to turbulent wind fluctuations within a simulated urban boundary layer. Their model simulations showed 2.5 µm and 10 µm particles to reach a maximum height of 0.04-0.06 meter above ground level (AGL), while 50 µm particles reached a maximum of 0.2 meter AGL and 75 µm particles reached at least 0.4 meter AGL, depending on friction velocity. Empirical analysis has shown that lift force is proportional to particle diameter to the power of approximately 1.5, so that large particles actually have larger initial vertical displacement than smaller particles. At the same time, lateral travel distance following resuspension tended to decrease linearly with increasing particle size, reflecting the counteracting force of gravity. For all cases simulated, the resuspension and deposition were estimated to occur over time frames on the order of seconds.

Early work described resuspension as an important process for wind erosion for particles up to 100 µm but indicated that particles larger than this rarely became suspended, and that the tendency of particles to remain airborne long enough for appreciable transport decreases sharply beyond a size of 10 to 20 µm (Nicholson, 1988; Gillette et al., 1974). As a result, long range transport of dust is usually limited to particles smaller than 10 µm (Prospero, 1999).

In urban environments the transport distance that must be traversed to penetrate indoors can be very short, and at the same time resuspension and dispersion of larger particles may be caused by motor vehicles. Resuspension of road dust by traffic becomes more

difficult with decreasing particle size because adhesive forces are stronger than shear force that is imparted by traffic-induced turbulent air movement (Harris and Davidson, 2008). The critical diameter at which resuspension occurs when a particle's settling velocity becomes lower than the friction velocity of air needed to move the particle from rest. The work of Gillette et al. (1974), in which a critical diameter of roughly 20 µm was estimated, is based on wind in an open landscape. It would be reasonable to expect that friction velocity would be higher for urban environments with traffic-induced turbulence (Britter and Hanna, 2003). Hence, it is possible that larger particles are resuspended in a heavily-trafficked urban setting (Nicholson and Branson, 1990).

Particle size determines the distance particles can travel and the height which they can achieve before they are removed by gravitational settling. Song and Gao (2011) observed that coarse mode Pb concentration was negatively correlated with wind speed (D_p = 14: ρ = -0.62; D_p = 7.8; ρ = -0.76), which suggests that coarse Pb may be dispersed by wind. Observations in near road environments indicate that roughly 15% of Pb in airborne dust in areas impacted by heavy traffic is greater than 10 µm (Cho et al., 2011; Lough et al., 2005; Zereini et al., 2005). Sabin et al. (2006b) also collected three size fractions greater than 11 µm and found that approximately 25% of all Pb mass was associated with particles larger than 29 µm at a site 10 meters from a freeway, but only a very small percentage of Pb mass was in this size fraction at an urban background site. These results suggest that both size distribution and concentrations in the immediate vicinity of roadways might differ from estimates based on concentrations from monitoring sites at some distance from roads or on elevated rooftops. In these studies, only one size fraction slightly greater than 10 µm was collected, but another study of road dust (not specific to Pb) reported size fractions extending up to 100 µm with a mass median diameter of greater than 60 µm (Yang et al., 1999). Although the Yang et al. (1999) study did not include Pb, the results suggest that resuspended dust can be larger than PM_{10}. Collectively, the size distribution of Pb-containing resuspended dust is uncertain.

Recent resuspension studies complement previous research indicating street dust half-lives on the order of one-hundred days (Allott et al., 1989), with resuspension and street run-off as major sinks (Vermette et al., 1991) as well as observations of a strong influence of street surface pollution on resuspension (Bukowiecki et al., 2010), observations of greater resuspension of smaller PM than coarser PM, leading to enrichment of metal concentrations in resuspended PM relative to street dust (Wong et al., 2006) and observations of wind speed, wind direction, vehicular traffic, pedestrian traffic, agricultural activities, street sweeping and construction operations as important factors determining resuspension.

2.3.2 Water

As described in the 2006 Pb AQCD (U.S. EPA, 2006b), atmospheric deposition, urban runoff, and industrial discharge have been identified as major sources of Pb in surface waters. Water columns have been described as transient reservoirs with Pb residence times in lakes typically several months long, and shorter residence times expected in turbulent waterways. Because dispersal in waterways is a relatively rapid process, concentrations in surface waters are highest near sources of pollution before substantial Pb removal by flushing, evaporation, and sedimentation occurs. Transport in surface water is largely controlled by exchange with sediments, and the cycling of Pb between water and sediments is governed by chemical, biological, and mechanical processes that are affected by many factors, including salinity, organic complexation, oxidation-reduction potential, and pH; ecological impacts of these factors are described in Section 6.4.9. As described in the 2006 Pb AQCD (U.S. EPA, 2006b), metals in waterways are transported primarily as soluble chelates and ions, or adsorbed on colloidal surfaces, including secondary clay minerals, iron (Fe) and manganese (Mn) oxides or hydroxides, and organic matter, and adsorption on organic or inorganic colloids is particularly important for Pb. The extent of sorption strongly depends on particle size as smaller particles have larger collective surface areas. Aqueous Pb concentrations also increase with increasing salinity. Pb is found predominantly as PbO or $PbCO_3$ in aqueous ecosystems. Pb is relatively stable in sediments, with long residence times and limited mobility. However, Pb-containing sediment particles can be remobilized into the water column. As a result trends in sediment concentration tend to follow those in overlying waters. Fe and Mn oxides are especially susceptible to recycling with the overlying water column. Although resuspension of sediments into overlying waters is generally small compared to sedimentation, resuspension of contaminated sediments is often a more important source than atmospheric deposition. Organic matter (OM) in sediments has a high capacity for accumulating trace elements. In an anoxic environment, removal by sulfides is particularly important. The following section highlights recent literature regarding the fate and transport of Pb in water systems. Section 6.2 synthesizes this information with ecosystem exposure data.

Runoff from storms was identified as a potentially important source of Pb in aquatic systems (U.S. EPA, 2006b). Runoff from atmospheric deposition, painted buildings, gutters, roofing materials and other housing materials were identified as major contributors to Pb in runoff waters. Investigations of building material contributions indicated runoff concentrations ranging from 2 to 88 mg/L, with the highest concentrations observed from more than 10-year-old paint and the lowest concentrations from residential roofs. There was some indication that Pb from roofing materials, siding, and piping could be due to dissolution of Pb carbonate (cerussite) or related compounds.

In several studies Pb in runoff was consistently mostly PM, with a relatively small dissolved fraction. Runoff release was dependent on storm intensity and length of dry periods between rain events, with greater runoff of Pb associated with more intense storms and with longer periods between rain events. Several studies indicated a "first flush effect," with highest runoff concentrations observed at the beginning of a rain event.

2.3.2.1 Pb Transport in Water and Sediment

Recent publications provide additional detail regarding Pb adsorption on Fe-rich and organic-rich colloids. Correlation between Pb concentration in unfiltered water with total Fe was observed (Hassellov and von der Kammer, 2008), which is consistent with previous research using cross flow filtration (Pokrovsky and Schott, 2002; Ross and Sherrell, 1999) and SEM examination of single particles (Taillefert et al., 2000).

Two distinct colloidal phases, one organic-rich (0.5-3 nm in diameter) and the other Fe-rich (>3 nm in diameter), have been observed to coexist in both soil isolates and river water (Stolpe and Hassellov, 2007). Pb was observed to be predominantly associated with Fe-oxide PM in river water but also associated with the organic colloids in the soil isolates (Hassellov and von der Kammer, 2008). Investigation of Pb binding onto ferrihydrite showed Pb binding data were consistent with Pb being held at the surface by sorption processes, rather than enclosed within the particle structure (Hassellov and von der Kammer, 2008).

Observations in boreal rivers and soil pore waters in permafrost dominated areas of Central Siberia indicated that Pb was transported with colloids in Fe-rich waters. Trace elements that normally exhibited limited mobility (including Pb) had 40-80% of their annual flux in the nominal dissolved phase, operationally defined as material that passes through a 0.45 µm pore-size filter, and that these metals had a higher affinity for organo-mineral Fe-Al colloids (Pokrovsky et al., 2006). Pokrovsky et al. (2006) postulated that during the summer, rainwater interacts with degrading plant litter in the top soil leading to the formation of Fe-Al-organic colloids with incorporated trace elements. Migration of trace element-Fe-Al-OM colloids may result in export of Pb and other elements to riverine systems. Most of the transport occurred after thawing had commenced. This contrasts with permafrost free areas where trace elements such as Pb are incorporated into Fe colloids during OM-stabilized Fe-oxyhydroxide formation at the redox boundary of Fe(II)-rich waters and surficial DOC-rich horizons. Similarly, during a spring flood (May) that exported 30-60% of total annual dissolved and suspended flux of elements including Pb, Pb was mainly in the nominal dissolved phase, operationally defined as

material that passes through a 0.45 μm pore-size filter (Pokrovsky et al., 2010). Pb adsorbed on colloidal surfaces rather than incorporated into particle structure is likely to be more readily dissolved because dissolution of the entire particle is not required.

Recent research on retention of Pb in water bodies and sediments has focused on the estuarine and marine environment, where considerable retention of Pb was observed in estuarine sediments. For a large riparian system, the Trinity River, Texas, Warnken and Santschi (2009) found that 80% of riverine Pb was retained in Lake Livingston, an estuarine region, while an additional 16% was removed to estuarine sediments, and only about 4% eventually reached the ocean. Geochemical (sorption by Fe oxyhydroxides), biological (seasonal uptake by sinking algae in Lake Livingston) and hydrological (dilution effects by increasing flow rates) processes were mainly responsible for controlling dissolved trace metal concentrations rather than pollution sources.

Overall, recent research on Pb transport in aquatic systems has provided a large body of observations confirming that Pb transport is dominated by Fe-rich and organic-rich colloids. In addition, new results indicated that although the 2006 Pb AQCD (U.S. EPA, 2006b) described rivers and lakes as temporary reservoirs with Pb lifetimes of months or less, estuaries can present a substantial barrier to transport into the open ocean.

2.3.2.2 Deposition of Pb within Bodies of Water and in Sediment

As described in the 2006 Pb AQCD (U.S. EPA, 2006b), in general Pb is relatively stable in sediments, with long residence times and limited mobility. As described in previous sections, Pb enters and is distributed in bodies of water largely in PM form. In rivers, particle-bound metals can often account for ≥ 75% of the total load, e.g., (Horowitz and Stephens, 2008). Areas near historically Pb emitting industries and urbanized areas tend to have greater aquatic Pb loads than areas more remote from Pb sources, as several studies have shown the strong positive correlation between population density and river or lake sediment Pb concentrations (Horowitz et al., 2008; Chalmers et al., 2007). Indeed, Chalmers et al. (2007) revealed that in river and lake sediments in New England, there was an order of magnitude difference between Pb sediment concentrations in rural versus urbanized areas.

The fate of Pb in the water column is determined by the chemical and physical properties of the water (pH, salinity, oxidation status, flow rate and the suspended sediment load and its constituents, etc). Desorption, dissolution, precipitation, sorption and complexation processes can all occur concurrently and continuously, leading to transformations and redistribution of Pb. The pH of water is of primary importance in determining the likely chemical fate of Pb in terms of solubility, precipitation or organic

complexation. In peatland areas, such as those in upland areas of the U.K., organic acids draining from the surrounding peatlands can lower stream water pH to below 4. Under these conditions, Pb-PM can be desorbed and released into solution, leading to elevated dissolved Pb concentrations (Rothwell et al., 2008). At the other end of the pH scale, Pb tends to remain or become complexed, precipitated or sorbed to suspended sediments in water, as observed by Das et al. (2008) who studied trace metal geochemistry in a South African lake with water pH of 9. They also found marked differences in Pb concentrations associated with increasing depth in the water column [e.g., the surface Pb-PM concentration of 2 µg/L increased to 60 µg/L at depth and the Pb concentration in the <0.45 µm fraction increased from 2 µg/L at the surface to 19 µg/L at depth (Das et al., 2008)]. This is suggestive of a settlement process in action.

In estuarine and wider marine environments the processes may be more complex because of the additional perturbation caused by tidal action and the strong effects of salinity. Again, PM forms of Pb are important in determining Pb distribution and behavior. Li et al. (2010a) reported that PM Pb accounted for 85 ± 15% and 50 ± 22% in Boston Harbor and Massachusetts Bay, respectively, while Lai et al. (2008b) reported a solid (acid soluble):dissolved Pb ratio of 2.6 for areas of the Australian sector of the Southern Ocean.

The accurate modeling of Pb behavior in marine waters (including estuaries) requires consideration of many parameters such as hydrodynamics, salinity, pH, suspended PM, fluxes between PM and dissolved phases (Hartnett and Berry, 2010). Several new advances in the study of Pb cycling in these complex environments have been described in recent publications. Li et al. (2010a) used particle organic carbon (POC) as a surrogate for the primary sorption phase in the water column to describe and model the partitioning of Pb between PM and dissolved forms. Huang and Conte (2009) observed that considerable change in the composition of PM occurs as they sink in the marine environment of the Sargasso Sea, with mineralization of OM resulting in increased Pb-PM concentration with increased depth. As a result of this depletion of OM in sinking particles, geochemical behavior at depth was dominated by inorganic processes, e.g., adsorption onto surfaces, which were largely independent of Pb source. Sinking rates in marine environments can vary, but a rate approximating 1 meter/day has been used in some models of Pb transport and distribution in aquatic-sediment systems (Li et al., 2010a). Surface sediment Pb concentrations for various continental shelves were collated and compared by Fang et al. (2009) (Table 2-2).

Table 2-2 Surface sediment Pb concentrations for various continental shelves.

Location	Digestion solution	Pb[a] (mg/kg)
East China Sea	$HCl/HNO_3/HF$	10-49 (27)[a]
Mediterranean, Israel coast	HNO_3	9.9-20
Aegean Sea	$HCl/HNO_3/HF$	21-44 (34)
Banc d'Arguin, Mauritania	$HCl/HNO_3/HF$	2.8-8.9
Campeche shelf, Gulf of Mexico	HCl/HNO_3	0.22-20 (4.3)
Laptev Sea, Siberia	$HCl/HNO_3/HF$	12-22
Pechora Sea, Russia	Not reported	9.0-22 (14)

[a]Values in parentheses are the average, where calculable

Source: Data from Fang et al. (2009) and references therein.

2.3.2.3 Flux of Pb from Sediments

Sediments can be either a source or a sink for metals in the aquatic environment. Release can be via re-suspension of the sediment bed via wind, wave and tidal action or by dissolution from sediment to the water column. When external Pb inputs to bodies of water are decreased by environmental improvement actions or regulations, contributions of Pb to the water column from the existing sediments can become an increasingly important source. Roulier et al. (2010) determined that Pb flux from sediments originated mostly from organic fractions, but also partially from Mn and Fe components undergoing reductive dissolution. The rate of release was controlled by OM content, particle size, clay type and content, and silt fraction (Roulier et al., 2010). The importance of sediment particle size, OM content and acid volatile sulfide concentration in relation to metal release was similarly identified (Cantwell et al., 2008); ecosystem effects related to sulfide concentration are described in Section 6.4.2.5. The effect of pH change on Pb release from lake sediments has also been examined, revealing that 1.8 protons (H^+) were exchanged per divalent metal cation released (Lee et al., 2008a). Processes governing Pb release from lake sediments, including microbial reductive dissolution of Fe, biogenic sulfide production and metal sorption-desorption, have been investigated in a basin heavily contaminated by historical precious metal mining activities, and results indicated that release of Pb from suboxic and anoxic zones of the heavily contaminated sediment act as a Pb source to the overlying water of the lake (Sengor et al., 2007). Bacardit and Camarero (2010a, b) performed a mass balance of Pb, Zn, and As for three lakes in the Central Pyrenees in France to identify dominant metals distribution processes. They estimated that flux from the catchment accounted for 91-99% of the lakes' Pb inputs,

while sediment flux accounted for 98-99% of Pb outputs. In this paper, sediment was only modeled as an output.

Disturbance of bed sediments also occurs by tidal action contributing to re-suspension of sediments. Benthic fluxes of dissolved metals released from sediments measured in Boston Bay were calculated as strong enough that in the absence of Pb inputs such benthic flux would reduce sediment Pb concentrations in Boston Bay to background levels in 30-60 years (Li et al., 2010a). In a related way, a half-life for sediment Pb (considering benthic flux alone as the loss mechanism) of 5.3 years was estimated for marine sediments off the Belgian coast (Gao et al., 2009). Atkinson et al. (2007) conducted experiments in an area contaminated by metal smelters, Lake Macquarie, Australia, to assess the factors that influence flux of metals from marine sediment. Low pH (pH = 6 ± 1), bioturbation, and other mixing processes were found to have stronger influence over flux than binding to sulfides, which were thought to be sequestered in deeper sediments.

Radakovitch et al. (2008) investigated the riverine transport of PM including Pb to the Gulf of Lion, France, and also concluded that a major part of annual fluxes could be delivered over a short time period. From budget calculations, riverine inputs were more important than atmospheric deposition and Pb concentrations in the prodelta sediments showed a strong correlation with OM content. These sediments, however, were not considered to be a permanent sink, as resuspension in these shallow areas was an important process. OM, Pb and other metals were enriched in resuspended PM compared with the sediment.

In a heavily contaminated, high salinity embayment upstream from Sydney Harbor in Australia, Birch and O'Hea (2007) reported higher total suspended solids, turbidity and total water metal concentration in surface compared with bottom water as well as a difference in suspended PM metal concentrations between surface water and bottom sediments, demonstrating that stormwater discharge was the dominant process of metal transfer during high rainfall events. Total suspended sediments (and total water metals) in bottom water were higher than in the surface water plume, indicating that resuspension of bottom sediment is a greater contributor of total suspended sediments than stormwater during such events, especially in shallower regions of the bay. Soto-Jimenez and Páez-Osuna (2010) determined diffusive and advective fluxes, geochemical partitioning of Pb and Pb-isotopic signatures in a study of mobility and behavior of Pb in hypersaline salt marsh sediments. They determined that sulfides were the main scavengers for Pb that was diagenetically released Pb.

Overall, recent research on Pb flux from sediments in natural waters provided greater detail on resuspension processes than was available in the 2006 Pb AQCD (U.S. EPA,

2006b), and confirms previous findings that resuspended Pb is largely associated with OM or Fe and Mn particles, but that anoxic or depleted oxygen environments in sediments play an important role in Pb cycling. This newer research confirms previous findings that resuspension and release from sediments largely occurs during discrete events related to storms. It has also confirmed that resuspension is an important process that strongly influences the lifetime of Pb in bodies of water.

2.3.2.4 Pb in Runoff

Runoff is a major source of Pb in surface waters. This complicates any evaluation of the contribution of atmospheric Pb to watersheds, which must take into account direct atmospheric deposition, runoff of atmospherically deposited Pb, and runoff of Pb from sources such as mine tailings or paint chips that are shed from outdoor structures. The 2006 Pb AQCD (U.S. EPA, 2006b) concluded that runoff was consistently mostly PM, with a relatively small dissolved fraction, and that dissolution of carbonate and related compounds were important contributors to Pb pollution in runoff waters. It also described Pb release into runoff as dependent on storm intensity and length of dry periods between rain events, and a "first flush effect," with highest runoff concentrations observed at the beginning of a rain event. Subsequent research has provided considerable new information about the flux of Pb from roadway and urban runoff and snow melt to watersheds.

Severe contamination due to export of anthropogenic Pb to adjacent ecosystems via urban runoff and domestic wastewater discharge and to a lesser extent by direct atmospheric deposition has been documented (Soto-Jiménez and Flegal, 2009). Recent investigations also confirm roof runoff as an important contributor to Pb pollution. Huston et al. (2009) measured Pb concentrations in water from urban rainwater tanks and found Pb concentrations in bulk deposition were consistently lower than in water in the rainwater tanks, but that sludge in the tanks had a high Pb content, indicating that not all major sources of Pb are from atmospheric deposition. Pb levels frequently exceeded drinking water standards. Pb flashing on the roofs was implicated as the source of Pb in the rainwater tanks although other possible sources include old paint and Pb stabilized PVC drain pipes (Lasheen et al., 2008; Weiss et al., 2006; Al-Malack, 2001).

New research has improved the understanding of suspended PM size ranges, speciation, and impacts of Pb runoff from urban soil and road dust. Soil and road dust have been identified as major sources of Pb pollution to near-coastal waters, leading to high Pb concentrations in stormwater runoff that became associated with dissolved and suspended

PM phases as well as bedload, material moved by rolling, sliding, and saltating along the bottom of a stream (Birch and McCready, 2009).

Several recent studies reported that the size distribution of Pb in PM transported in runoff is relatively uniform. Characterization of the roadside dust in Australia showed that Pb in PM was approximately uniformly distributed among PM size fractions of up to 250 µm. The Pb-containing particles had the potential to be dispersed to some distance into sensitive ecosystems (Pratt and Lottermoser, 2007). Pb in roadside dusts in Thessaloniki, Greece was characterized by Ewen et al. (2009) and no difference in Pb concentration was found between <75 µm and 75-125 µm PM size ranges, although a difference in the chemical form of Pb between slightly versus highly contaminated areas was observed.

Ewen et al. (2009) reported that Pb was mainly in a more exchangeable form (similar to that in an old auto-catalyst reference material) in small particles, but in the residual, or least mobile fraction in larger particles. In urban road dust from Manchester U.K., Pb-bearing Fe-oxides were observed to be dominant in most of the size fractions, and $PbCrO_4$ comprised 8-34% of total Pb with the highest concentrations being found in the largest and smallest size fractions. $Pb(CO_3)_2$ and $Pb(OH)_2$ were measured in the two middle size fractions, while PbO and $PbSO_4$ were present in the largest and smallest size fractions (Barrett et al., 2010).

Murakami et al. (2007) also emphasized the importance of $PbCrO_4$ as an important species of Pb in runoff from road surfaces. That study identified individual particles containing high levels of Pb and Cr (≥ 0.2%), most likely from the yellow road line markings. The identified PM constituted 46% of Pb in heavy traffic dust and 7-28% in dust from residential areas and soakaway sediments. The presence of such particles in soakaway sediments is consistent with their low environmental solubility.

Recent research also continues to document the first flush effect described in the 2006 Pb AQCD. Flint and Davis (2007) reported that in 13% of runoff events, more than 50% of Pb was flushed in the first 25% of event water. A second flush occurred less frequently (4% of runoff events for Pb). In agreement with the 2006 Pb AQCD (U.S. EPA, 2006b), most recent studies have concluded that, during storm events, Pb is transported together with large PM. Some studies, however, found that Pb was concentrated in the fine PM fraction and, occasionally, Pb was found predominantly in the dissolved fraction. Tuccillo (2006) found that Pb was almost entirely in the >5 µm size range and, indeed, may be associated with PM larger than 20 µm. (Sansalone et al., 2010) compared Pb-containing PM size distributions from Baton Rouge, LA, New Orleans, LA; Little Rock, AR; North Little Rock, AR; and Cincinnati, OH and found no common distribution pattern. Pb was associated with Cincinnati PM mainly in the <75 µm fractions, at Baton Rouge and Little Rock Pb mainly in the 75-425 µm PM

fractions, and at North Little Rock Pb predominantly in the >425 µm PM fractions. New Orleans Pb was almost uniformly distributed among the smaller size PM fractions. McKenzie et al. (2008) found that Pb was enriched in the finest PM (0.1-0.3 µm) in stormwater samples collected in California, particularly for storms that occurred during and after an extended dry period.

Guo et al. (2006a) investigated the effect of engineered partial exfiltration reactor (PER) systems on the partitioning and speciation of Pb in rainfall-runoff at the upstream end of an urban source area catchment that is part of the much larger urbanized and industrial Mill Creek watershed in Hamilton County, Ohio. The catchment is paved to a large extent with asphalt and is used for transportation. Guo et al. (2006a) investigated a catchment that drained toward a wide grassy area and found that Pb was mainly associated with dissolved organic matter (DOM) in the influent. The study suggested that interaction of the rainfall-runoff with the grassy area may have resulted in removal of PM-bound Pb. PM amount and size can also be influenced by the runoff surface. Guo et al. (2006a) found that Pb entering the engineered PER system was mainly in the dissolved, rather than particulate, fraction of the rainfall runoff with ~76%.

There were several recent observations of a relationship between road traffic volume and runoff Pb concentration, although a clear relationship was not always observed. At a relatively clean location, Desta et al. (2007) studied highway runoff characteristics in Ireland and found that although as expected, total Pb concentration was strongly correlated with total suspended solids, no relationship between total suspended solids and rainfall, rain intensity, antecedent dry days or runoff event duration were observed. They concluded that runoff composition from site to site could be highly variable. Most other studies did find a relationship between traffic volume and Pb concentration. A California study of highway runoff by Kayhanian et al. (2007) reported that 70-80% Pb was in particulate form for both non-urban and urban highways, and that the concentration of Pb in runoff from low traffic flow (30,000-100,000 vehicles/day) urban highways was 50% higher than that from non-urban highways (total Pb mean = 16.6 µg/L). Additionally, the concentrations in runoff from high traffic flow (>100,000 vehicles/day) urban areas were five times higher than those from non-urban highways. Helmreich et al. (2010) characterized road runoff in Munich, Germany, with an average daily traffic load of 57,000 vehicles. The mean total Pb concentration, 56 µg/L (maximum value = 405 µg/L), lay in between the values for low traffic flow and high traffic flow runoff from urban areas in California, i.e., there was good agreement with Kayhanian et al. (2007). There was no detectable dissolved Pb, i.e., 100% in PM form. Seasonal effects of highway runoff have also been observed recently. Hallberg et al. (2007) found that summer particle-bound Pb concentrations in runoff water in Stockholm ranged from 1.37-47.5 µg/L while, in winter, the range was 1.06-~296 µg/L. There was a strong

correlation between Pb (and most other elements) and total suspended solids (R^2 = 0.89). Helmreich et al. (2010) also found higher metal concentrations during cold seasons in Stockholm but Pb concentrations increased only slightly during the snowmelt season. There was no change in the distribution of Pb between dissolved and PM forms for the rain and snowmelt periods. Runoff from urban snowmelt has been intensively investigated since the 2006 Pb AQCD was published. The relocation of snow means that the area receiving the snowmelt is not necessarily the same area that received the snowfall. Magill and Sansalone (2010) also noted that plowed snowbanks alongside roadways form a temporary linear reservoir for traffic generated constituents such as metals and PM. Snowmelt concentrations of metals such as Pb can therefore be several orders of magnitude higher than those in rainfall runoff (Sansalone and Buchberger, 1996). The melt process usually occurs in a sequence: pavement melt, followed by roadside (impervious) and finally pervious area melt. As part of this sequence, rain-on-snow can transport high loads of PM-associated pollutants (Oberts, 2000). Westerlund and Viklander (2006) investigated differences in PM and Pb concentrations between rainfall events occurring during snowmelt and rain periods. Runoff events occurring during the snowmelt period (i.e., rain-on-snow) had about five times higher numbers of particles (in the size range 4 to 120 μm)/Liter of runoff. The first rain-on-snow event was characterized by an increase in the number of particles in the 4 to 25 μm size range. The rain-on-snow gave a "flush" through the snow but this was still not sufficient to transport the larger sized particles. Only the highest energy rain-on-snow events increased transport of PM across the entire size spectrum. There was no difference in particle size distributions between snowmelt and rain on snow events, although more was transported during snowmelt. Pb concentrations were most strongly associated with the smaller PM size fractions.

Overall, there was a significant difference between the melt period and the rain period in terms of concentrations, loads, transportation and association of heavy metals with particles in different size fractions (Westerlund and Viklander, 2006). Over a 4-year period, Magill and Sansalone (2010) analyzed the distribution of metal in snow plowed to the edge of roads in the Lake Tahoe catchment in Nevada, and concluded that metals including Pb were mainly associated with large particles (179-542 μm). The PM-associated metal could be readily separated from runoff water (e.g., in urban drainage systems), but there is potential for leaching of metals from the PM within storage basins (Ying and Sansalone, 2008). For adsorbed species that form outer sphere complexes, a decrease in adsorption and an increase in aqueous complexes for pollutant metals is a likely consequence of higher deicing salt concentrations. If metals form inner-sphere complexes directly coordinated to adsorbent surfaces, background deicing salt ions would have less impact. It is thought that physical and outer-sphere complexes predominate for

coarse PM, as was the case in Nevada, and so leaching would be likely to cause an increase in dissolved phase Pb concentrations.

Rural runoff has also been extensively studied since publication of the 2006 Pb AQCD (U.S. EPA, 2006b), including several recent publications on a forested watershed (Lake Plastic) in central Ontario (Landre et al., 2010, 2009; Watmough and Dillon, 2007) and nearby Kawagama Lake, Canada (Shotyk and Krachler, 2010). Results indicated that bulk deposition substantially decreased to 0.49 mg/m^2 in 2002 from 1.30-1.90 mg/m^2 in 1989-91. The upland soils retained >95% of the Pb in bulk deposition, i.e., leaching losses to stream water were small. The wetland area was, however, a net source of Pb with annual Pb concentrations in stream water ranging from 0.38 to 0.77 µg/L. Lake sediments were efficient sinks for atmospherically deposited Pb with 80-91% of the Pb input being retained. Up to 68% of the Pb entering the lake was derived from the terrestrial catchment. Overall, the watershed effectively retained atmospherically deposited Pb, but some Pb was then redistributed from the catchment to the lake sediments; and the Pb in the near-surface lake sediments reflected terrestrially transported soil material, rather Pb being deposited from the atmosphere. The highest concentrations of dissolved organic carbon (DOC), Fe and Pb in the wetland draining stream occurred in summer when Pb concentration frequently exceeded 1 µg/L (Landre et al., 2009).

Graham et al. (2006) observed two temporally separated mechanisms occurring during storm events in a rural organic rich upland catchment. At the beginning of an event, Pb was transported together with large particles in the >25 µm size range, but after several hours Pb was mainly transported with colloidal or DOM (<0.45 µm), and the remaining 30-40% of storm related Pb was transported in this form. This indicated that overland flow rapidly transported Pb-PM into the receiving streams at the very beginning of the event, and this was followed within a few hours by transport of organic-colloidal Pb via near-surface throughflow. The authors used a conservative estimate of Pb removal, based on their observations that the catchment was continuing to act as a sink for Pb. These observations about the transport and fate of Pb agree well with those of Watmough and Dillon (2007) and Shotyk et al. (2010).

Soil type was also found to have a strong influence on runoff contributions. Dawson et al. (2010) found that for organic-rich soils Pb was mobilized from near-surface soils together with DOC, but for more minerogenic soils percolation of water allowed Pb bound to DOC to be retained in mineral horizons and combine with other groundwater sources. The resulting Pb in stream water had a more geogenic signature (Dawson et al., 2010). The findings of both Graham et al. (2006) and Dawson et al. (2010) were important because the provenance and transport mechanisms of Pb may greatly affect the net export to receiving waters.

In another study, Rothwell et al. (2007b) observed stormflow Pb concentrations in a peat catchment in Southern Pennines, U.K. almost three times higher than those reported by Graham et al. (2006) for northeastern Scotland. The generally high dissolved Pb were due to high soil Pb pools and high stream water DOC concentrations (Rothwell et al., 2007b). In a separate study, Rothwell et al. (2007a) showed that OM was the main vector for Pb transport in the fluvial system. Some seasonal variability was observed: declining Pb concentrations in autumn stormflow may indicate the exhaustion of DOC from the acrotelm (the hydrologically active upper layer of peat which is subject to a fluctuating water table and is generally aerobic) or a dilution effect from an increasing importance of overland flow.

Erosion of agricultural soils and the effects of different types of storm events on soil particle and Pb losses from these soils was characterized by Quinton and Catt (2007). A close link between metal concentration and the silt, or clay and organic content of stream sediments was consistent with enrichment of metals as a consequence of small erosion events. They also noted that short intense events could produce the same amount of sediment as longer low-intensity events. More intense events, however, could mobilize a wider range of particle sizes whereas low intensity events mobilized finer but more metal-rich material. Smaller events accounted for 52% of Pb losses from the agricultural soil.

The Tinto River in Spain drains one of the largest polymetallic massive sulfide regions in the world: the Iberian Pyrite Belt. Evaporitic sulfate salts, formed as a result of acid mine drainage processes, are considered to be a temporary sink for many heavy metals. Upon the arrival of rainfall, however, they rapidly dissolve, releasing acidity and contaminant metals into receiving waters. Thus rivers in semi-arid climate regions such as the Tinto River which alternate between long periods of drought and short but intense rainfall events, can experience quick acidification and increases in metal concentration. In a study of such events, Cánovas et al. (2010) found that while many element concentrations decreased during events, the concentrations of Fe, Cr, Pb and As increased. This was attributed to the redissolution and transformation of Fe oxyhydroxysulfates and/or desorption processes.

Dunlap et al. (2008) studied a large (>160,000 km^2) riparian system (the Sacramento River, CA) and showed that the present day flux of Pb into the river was dominated by Pb from historical anthropogenic sources, which included a mixture of high-ratio hydraulic Au mining-derived Pb and persistent historically-derived Pb from leaded on-road gasoline. Outside of the mining region, 57-67% of Pb in suspended colloidal material was derived from past on-road gasoline emissions and 33-43% was from hydraulic Au mining

sediment. Periods of high surface runoff mobilize additional fluxes of Pb from these two sources and carry them into the river.

Rothwell et al. (2007b) commented that although there have been substantial reductions in sulfur deposition to U.K. uplands over the last few decades (Fowler et al., 2005), anthropogenic acidification of upland waters is still possible if there is nitrogen leaching from the surrounding catchment and this may increase with nitrogen saturation (Curtis et al., 2005). Rothwell et al. (2007b) predicted that if an increase in surface water acidification is coupled with further increases in DOC export from organic-rich catchments, metal export from peatland systems will increase. The deterioration of peat soils by erosion is considered to be exacerbated by climatic change. Rothwell et al. (2010) used digital terrain analysis to model suspended Pb concentrations in contaminated peatland catchments. The peat soils of the Peak District are characterized by extensive eroding gullies and so they were combined in an empirical relationship between sediment-associated Pb concentrations and mean upslope gully depth with fine-resolution mapping of the gully areas. This model will enable prediction of metal contamination in receiving waters.

Klaminder et al. (2010) investigated the environmental recovery of sub-arctic lakes in response to reduced atmospheric deposition over the last few decades. They found that there had been no improvement in surface sediments and indeed the reduction in Pb contamination had been much less than the 90% reduction in emissions over the last four decades. The weak improvement in the $^{206}Pb/^{207}Pb$ ratio together with the Pb contaminant concentrations suggests that catchment export processes of previously-deposited atmospheric contaminants have had a considerable impact on the recent contaminant burden of sub-arctic lakes. In Arctic regions, soil export of contaminants to surface waters may dramatically increase in response to climate change if it triggers thawing of frozen soil layers. It is thought that thawing may generate accelerated soil erosion, altered hydrological flow paths, increased runoff and exposure of soluble compounds that had previously been in the frozen layers. At this stage, however, the links between catchment export and climate change have not yet been clearly established.

Coynel et al. (2007) also considered the effects of climate change on heavy metal transport. In this case, the scenario of flood-related transport of PM in the Garonne-Gironde fluvial-estuarine system was investigated. Export of suspended PM during a five-day flood in December 2003 was estimated at ~440,000 tons, accounting for ~75% of the annual suspended PM fluxes. Sediment remobilization accounted for ~42% of the total suspended particulate matter (SPM) flux during the flood event (~185,000 tons suspended PM) and accounted for 61% of the 51 tons Pb that was exported. Coynel et al. (2007) postulate that flood hazards and transport of highly polluted sediment may

increase as a result of climate change and/or other anthropogenic impacts (flood management, reservoir removal).

In heavily contaminated catchments [e.g., that of the Litavka River, Czech Republic (Zak et al., 2009)], the flux of heavy metals to the river during storm events can be substantial. Even during a minor 4-day event, 2,954 kg of Pb was transported, and the majority was associated with suspended PM. For the Adour River in a mountainous area of France, Pb pollution predominantly originated from mining activities, and Point et al. (2007) showed that 75% of annual soil fluxes into the river were transported in 30-40 days.

The consequences of flood management (dam flushing) practices on suspended PM and heavy metal fluxes in a fluvial-estuarine system (Garonne-Gironde, France) were considered by Coynel et al. (2007). Dam flushing enhanced mobilization of up to 30-year-old polluted sediment from reservoir lakes. Sediment remobilization accounted for ~42% of the total suspended PM fluxes during the flood and strongly contributed to PM-bound metal transport (61% for Pb). They concluded that flood management will need to be taken into consideration in future models for erosion and pollutant transport.

Bur et al. (2009) investigated the associations of Pb in stream-bed sediments of the French Gascony region. They found that Pb enrichment in stream sediments was positively correlated with catchment cover and increasing organic content whereas Pb concentration was strongly linked with Fe-oxide content in cultivated catchments. For the low-OM, anthropogenic Pb was associated with carbonates and Fe-oxides (preferentially, the amorphous fraction). Fe-oxides became the most efficient anthropogenic Pb trapping component as soon as the carbonate content is reduced. They noted, however, that OM was always weakly involved. N'Guessan et al. (2009) also studied trace elements in stream-bed sediments of the French Gascony region. They used enrichment factors to show that only ~20-22% of Pb was from anthropogenic sources with the remainder originating from natural weathering processes.

Overall, research results from the last several years have greatly expanded the extent of the knowledge concerning Pb from runoff. Substantial Pb input to estuarine and marine ecosystems has been well documented. More detail concerning the origin of Pb from roof runoff has led to the conclusion that roof flashing could be especially important. Research on road runoff has provided valuable insight into the size and composition of suspended sediment particles, indicating that size distributions for Pb-containing PM in runoff water vary from study to study and from location to location. Recent studies confirmed the "first flush" effect, releasing more Pb at the beginning of rainfall than subsequently, and documented size distributions of Pb-containing PM also vary considerably when water from the first flush is isolated. Influence of road traffic volume on runoff has also been more fully documented in recent years. The role of urban snowmelt and rain-on-snow

events is also better understood, and it has been observed that greater runoff occurs from snowmelt and in rain-on-snow events than when snow is not present, and that metals, including Pb, are often associated with coarse PM under these circumstances. Runoff in rural areas is strongly controlled by soil type and the presence of vegetation with less runoff and greater retention in mineral soils. When grass is present, there is more runoff for soils high in OM. Runoff also follows a two-step process of transport of larger particles at the beginning of an event, followed within hours by transport of finer colloidal material. Some initial research on the effects of climate change on runoff has focused on documenting the association between increased runoff and more intense rain events and greater thawing. Overall, recent research has provided greater detail on amounts, particle size distributions, composition, and important processes involving Pb transport. The understanding of Pb runoff has become more complete since publication of the 2006 Pb AQCD (U.S. EPA, 2006b) (U.S. EPA, 2006b).

2.3.3 Soil

The 2006 Pb AQCD (U.S. EPA, 2006b) summarized that Pb has a relatively long retention time in the organic soil horizon, although its movement through the soil column also suggests potential for contamination of groundwater. Leaching was consistently observed to be a slower process for Pb than for other contaminants because Pb was only weakly soluble in pore water, but anthropogenic Pb is more available for leaching than natural Pb in soil. Pb can bind to many different surfaces and Pb sorption capacity is influenced by hydraulic conductivity, solid composition, OM content, clay mineral content, microbial activity, plant root channels, animal holes, and geochemical reactions. As a result of Pb binding to soil components, leaching is retarded by partitioning to soils, which is not only influenced by sorption capacity, but leaching also increases with proximity to source, increasing pH, and increasing metal concentrations. Leaching is also strongly influenced by pore water flow rates, with more complete sorption contributing to slower leaching at lighter flows. Leaching rates are especially high in soils with a high Cl content, but typically the most labile Pb fraction is adsorbed to colloidal particles that include OM, clay, and carbonates. Transport through soils is enhanced by increasing the amount of colloidal suspensions, increasing colloidal surface charge, increasing organic content of colloids, increasing colloidal macroporosity, and decreasing colloidal size. Acidity and alkalinity have a more complex influence, with sorption maximized at neutral pH between pH = 5 and pH = 8.2, and greater mobility at higher and lower pH. High Pb levels have been observed in leachates from some contaminated soils, but this effect appears to be pH dependent. In several studies of contaminated soils a substantial

fraction of Pb was associated with Mn and Fe oxides or carbonate. Influence of soil chemistry on Pb effects in ecosystems is described in Section 6.3.2.

2.3.3.1 Deposition of Pb onto Soil from Air

As described in the 2006 Pb AQCD (U.S. EPA, 2006b), a considerable amount of Pb has been deposited from air onto soils in urban areas and near stationary sources and mines. Removal and translocation of Pb in soil is an ongoing process.

High geometric mean Pb soil concentrations (for soil particles between 180 μm and 2 mm in size: 51 ppm; for soil particles smaller than 180 μm in size: 105 ppm) were observed near stationary sources such as smelters and battery disposal operations, and soil Pb concentrations decreased rapidly with distance from the source. Several recent studies continue to document high concentrations of Pb in soil. A study of soil Pb concentrations in Queensland, Australia described atmospheric transport and deposition of Pb in soils (due to ongoing emissions from nearby mining [which began in 1924] and smelting [which began in 1931] activities) are continuing to impact the urban environment, resulting in elevated soil Pb concentrations at urban property sites within 2 km of the mines (Taylor et al., 2010). Similarly, sediment cores from four remote Canadian Shield headwater lakes located along a transect extending 300 km from a non-ferrous metal smelter generated useful information about distance of Pb transport from the smelter prior to deposition (Gallon et al., 2006). Shotyk and Krachler (2010) postulated that long-range transport of Pb from a smelter at Rouyn-Noranda may still contribute to deposition on these lakes. Recent measurements of deposition fluxes to soil in rural and remote areas have ranged from approximately 0.5 mg/m^2 per year to about 3 mg/m^2 per year with fair agreement between locations in Canada, Scandinavia, and Scotland. These measurements showed a substantial decrease compared to when leaded on-road gasoline was in widespread use (Shotbolt et al., 2008; Watmough and Dillon, 2007; Fowler et al., 2006; Graham et al., 2006).

There has been considerable interest in the response of soils to the decreasing aerosol Pb concentrations and Pb deposition rates that have been recorded in recent years. Kaste et al. (2006) resampled soils at 26 locations in the Northeast U.S. (during a 2001-2002 survey of soil sites originally sampled in 1980), and found no significant change in the amount of Pb in the O-horizon at high altitude sites, suggested to be related to reduced microbial activity at altitude. However, the amount of Pb in the O-horizon had decreased at some locations in the southern part of the survey region (Connecticut, New York, Pennsylvania), where the forest soils have typically thinner O-horizons, the reasons for which are discussed further in Section 2.3.3.2. Relatively higher Pb concentrations were

also found among sampled areas of Japan with differing geology (Takamatsu et al., 2010).

Further support for the use of mosses as bioindicators or monitors for atmospheric Pb inputs to peat bogs have recently been published by Kempter et al. (2010) who found that high moss productivity did not cause a dilution of Pb concentrations in peat bogs. They also found that productive plants were able to accumulate particles from the air and that rates of net Pb accumulation by the mosses were in excellent agreement with the atmospheric fluxes obtained by direct atmospheric measurements at nearby monitoring stations. In addition, Bindler et al. (2008) used Pb isotopes to compare the distribution of Pb in the forest soils with that of lake sediments where no "plant pumping" processes could be invoked, and used Pb isotope ratios to demonstrate that observations were consistent with anthropogenic Pb deposition to the soils rather than intermixing of natural Pb from underlying mineral soil horizons.

Overall, recent studies provided deposition data that were consistent with deposition fluxes reported in the 2006 Pb AQCD (U.S. EPA, 2006b), and demonstrated consistently that Pb deposition to soils has decreased since the phase-out of leaded on-road gasoline. Follow-up studies in several locations at high altitude indicated little change in soil Pb concentrations since the phase-out of leaded on-road gasoline, although reductions in surface soil Pb concentrations have been documented in some areas.

2.3.3.2 Sequestration of Pb from Water to Soil

The 2006 Pb AQCD described Pb as being more strongly retained in soil than other metals because of its weak solubility in pore water, but that anthropogenic Pb was more available for leaching than natural Pb (U.S. EPA, 2006b). It also described a complex variety of factors that influence Pb retention, including hydraulic conductivity, solid composition, OM content, clay mineral content, microbial activity, plant root channels, geochemical reactions, colloid amounts, colloidal surface charge, and pH.

Recent research in this area has provided more insight into the details of the Pb sequestration process. Importance of leaf litter was further investigated, and it was observed that the absolute Pb content can be substantial because rain events cause splashing of the leaf litter with soil thus placing the litter in direct contact with soil metals. The resulting increase in leaf litter metal concentrations suggests that the litter can act as a temporary sink for metals from the soil around and below leaves on the ground. The low solubility of Pb in the leaf litter indicates that the Pb is tightly bound to the decomposing litter, making the decomposing leaves act as an efficient metal storage pool (Scheid et al., 2009). Differences between throughfall (i.e., water depositing onto the

soil following collection on leaves) and litterfall (i.e., deposition of leaves, bark, and other vegetative debris onto soil) in forested areas have been investigated in forested areas, and the combined input of Pb to the forest floor from throughfall and litterfall was approximately twice that measured in bulk deposition (Landre et al., 2010). The difference was attributed to a substantial contribution from internal forest cycling and indicates that bulk deposition collectors may underestimate the amount of Pb reaching the forest floor by about 50% (Landre et al., 2010).

New research has also provided details about the complexity of Pb sequestration during soil OM decomposition. Schroth et al. (2008) investigated Pb sequestration in the surface layer of forest soils and the transformation of Pb speciation during soil OM decomposition. The pH range for forest floor soils in the Northeast U.S. is typically 3.5-5 and, under these conditions, dissolved Pb would adsorb strongly to soluble OM and to Fe/Al/Mn oxides and oxyhydroxides. It had been thought that the high affinity of Pb for organic ligands meant that sequestered atmospheric Pb would be preferentially bound to soluble OM. As a consequence, decomposition of OM would lead to Pb migration to the underlying mineral layers where it would be precipitated with the dissolved OC or adsorbed to pedogenic mineral phases. However, recent research has revealed a more complicated picture of gasoline-derived Pb associations in the forest floor. More recent research indicates that, as decomposition progresses, Pb and Fe become more concentrated in “hotspots” and Pb likely becomes increasingly distributed on surfaces associated with Fe and Mn (and to some extent Ca). It was postulated that Pb was initially bound to labile organic sites but, following decomposition, the Pb was adsorbed at reactive sites on pedogenic mineral phases (Schroth et al., 2008). Differences in litter types were also reported, with more rapid decomposition of OM in deciduous litter mobilizing more Pb initially bound to labile OM than coniferous litter, and producing more pedogenic minerals that could readily sequester the released Pb (Schroth et al., 2008). In the next stage of the study, the speciation of Pb in the O-horizon soils of Northern Hardwood, Norway spruce and red pine forest soils were compared. In general there was good agreement between the Pb speciation results for the soils and those for the laboratory decomposition experiments. Specifically, for the Northern Hardwood forest soil, a little more than 60% of the Pb was bound to soil organic matter (SOM) and this percentage increased to ~70% and ~80% for the Norway spruce and red pine soils, respectively. In all three cases, however, most of the remainder of the Pb was bound to ferrihydrite rather than to birnessite. This was not considered to be surprising because of the well-known leaching and cycling behavior of Mn that would be expected in the natural system. Thus the prevalence of Mn phases in the field based samples would be lessened (Schroth et al., 2008).

More generally, other studies have observed Pb sorption to Mn and Fe phases in soils. For example, Boonfueng et al. (2006) investigated Pb sequestration on Mn oxide-coated montmorillonite. Pb formed bidentate corner-sharing complexes. It was found that Pb sorption to MnO_2 occurred even when MnO_2 was present as a coating on other minerals, e.g., montmorillonite. Although their importance in the near-surface phases has clearly been demonstrated by Schroth et al. (2008), ferrihydrite surfaces may not be a long-term sink for Pb since reductive dissolution of this Fe(III) phase may release the surface-bound Pb into the soil solution. Sturm et al. (2008) explored the fate of Pb during dissimilatory Fe reduction. Pb was indeed released but was then incorporated into less reactive phases. These phases could not, however, be identified. Even so, it was asserted that Pb should be largely immobile under Fe-reducing conditions due to its incorporation into refractory secondary minerals.

Kaste et al. (2006) found that Pb species currently in the soil O-horizons of the Northeast U.S. differed considerably from those that were originally deposited from fossil fuel combustion (including on-road gasoline). $PbSO_4$ was considered to be the main form of Pb that had been delivered from the atmosphere to the surface of the Earth and it was postulated that the presence of sulfate may have facilitated the adsorption of Pb to colloidal Fe phases within the organic-rich horizons.

Altogether, these new results enhance the understanding of Pb sequestration in forest soils. First, the role of leaf litter as a major Pb reservoir is better understood. Second, the effect of decomposition on Pb distribution and sequestration on minerals during OM decomposition has been further characterized, and finally, the relative importance of Mn and Fe in sequestration is better understood.

Recent research also addressed roadside soils. Jensen et al. (2006) found that Pb was retained by an organic-rich blackish deposit with a high OM content and elevated soil Pb concentrations, originating from total suspended solids in road runoff and from aerial deposition. Hossain et al. (2007) observed that after long dry periods, OM oxidation may potentially result in the release of Pb. Microbial activity may also breakdown OM and have similar consequences (i.e., Pb release). Bouvet et al. (2007) investigated the effect of pH on retention of Pb by roadside soils where municipal solid waste incineration (MSWI) bottom ash had been used for road construction. They found that the Pb that had leached from the road construction materials was retained by the proximal soils under the prevailing environmental conditions (at pH = 7, <2% was released, but at pH = 4, slightly more Pb (4-47%) was released) and the authors speculated that the phase from which Pb had been released may have been $Pb(CO_3)_2(OH)_2$, indicating that sequestration of Pb via formation of oxycarbonate minerals is only effective at near-neutral to alkaline pH values (Figure 2-12 in Section 2.3.3.3).

Other recent research on Pb sequestration focused on microbial impacts and soil amendments. There have been few if any previous observations of microbial sequestration of Pb in soil. Perdrial et al. (2008) observed bacterial Pb sequestration and proposed a mechanism of Pb complexation by polyphosphate. They also postulated that bacterial transport of Pb could be important in sub-surface soil environments. Wu et al. (2006) also concluded that Pb adsorption to the bacterial cell walls may be important with respect to Pb transport in soils. This new area of research suggests that bacteria can play an important role in both sequestration and transport of Pb. Phosphate addition to immobilize Pb-contaminated soils has often been used to immobilize Pb in situ through the formation of Pb phosphate minerals such as chloropyromorphite. Recent research investigated factors affecting the long-term stability of such products, which depends on the equilibrium solubility and the dissolution rate of the mineral, trace impurities, such as $Pb(OH)_2$, the presence of complexing agents, and pH (Xie and Giammar, 2007). Overall, in agreement with the 2006 Pb AQCD (U.S. EPA, 2006b), the addition of phosphate can enhance immobilization of Pb under certain conditions in the field but may cause desorption and mobilization of anionic species of As, Cr and Se.

2.3.3.3 Movement of Pb within the Soil Column

The 2006 Pb AQCD summarized studies that demonstrated that Pb has a long retention time in the organic soil horizon; it also has some capacity to leach through the soil column and contaminate groundwater more than other contaminants do, because Pb is only weakly soluble in pore water (U.S. EPA, 2006b). The fate of any metal transport in soil is in response to a complex set of parameters including soil texture, mineralogy, pH and redox potential, hydraulic conductivity, abundance of OM and oxyhydroxides of Al, Fe, and Mn, in addition to climate, situation and nature of the parent material. As a consequence, it is impossible to make general conclusions about the final fate of anthropogenic Pb in soils. Indeed, Shotyk and LeRoux (2005) contend that the fate of Pb in soils may have to be evaluated on the basis of soil type. Some generalizations are, however, possible: Pb migration is likely to be greater under acidic soil conditions (Shotyk and Le Roux, 2005). In this respect, it would be expected that there should be considerable mobility of Pb in the surface layers of certain types of forest soils. This section reviews recent research on movement of Pb through soil types by first focusing on forest soils, followed by a broader treatment of a more diverse range of soils.

Forest Soils and Wetlands

Several studies confirmed the slow downward movement of Pb within the soil column. Kaste et al. (2006) found that the amount of Pb in O-horizon soils had remained constant at 15 of 26 sites in remote forested areas of the Northeast U.S. that had been re-sampled after a 21-year time period had elapsed, but that measured soil Pb concentrations were lower than predicted concentrations from total deposition, strongly suggesting that the O-horizon had not retained all of the atmospheric Pb, and that a proportion of the atmospheric deposition must have leached into the underlying mineral layers. At some sites, mainly those at the southern latitudes and lower altitude sites, the proportion of Pb that had been leached downward from the O-horizon was quite considerable. Relative retention of Pb was influenced by the rate of OM decomposition, depth of soil O-horizon, and pH. For soils where Pb was strongly retained by the O-horizon, a relationship between Pb and Fe-rich phase was observed, but Pb was also significantly correlated with other metals. XANES data suggested a possible interaction with an amorphous Fe oxide, but spectra were not entirely explained by Fe and oxygen and an additional spectral feature suggested the presence of a sulfur (S) or phosphorus (P) atom, which could result if OM functional groups were binding to Pb. Kaste et al. (2006) concluded that a substantial fraction of Pb was associated with amorphous Fe-hydroxides. The strong binding of Pb coupled with the low solubility of Fe phases under oxic conditions, helped to explain the relatively long residence time of gasoline-derived Pb in forest floors which had thick O-horizons and were well-drained. In the situations where Pb was leached downward to a large extent, mobility was likely governed by OM decomposition and colloidal transport of Pb associated with colloidal Fe and OM.

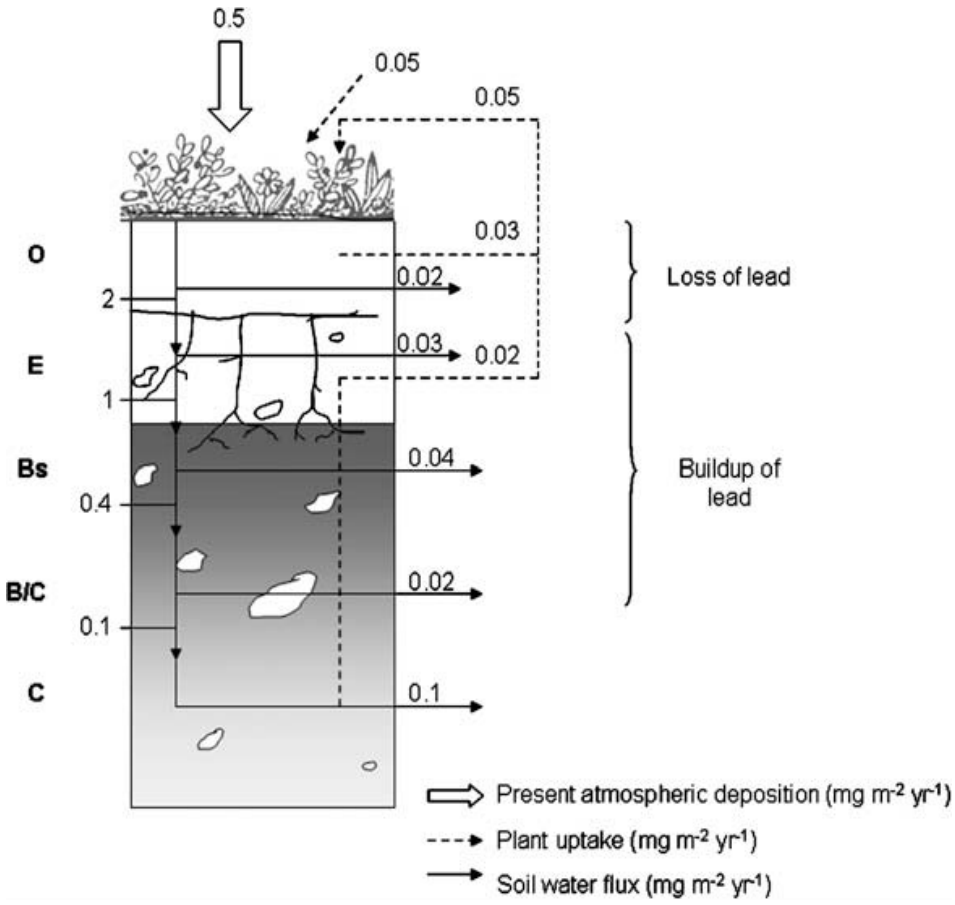
Klaminder et al. (2006b) also considered the transfer of Pb from the O-horizon to the underlying mineral horizons (including the C-horizon). They concluded that atmospheric pollution-derived Pb migrated at a rate about 10-1,000 times slower than water. They assumed that Pb was mainly transported by dissolved OM and so the mean-residence-time of Pb in the O-horizon depended on OM transport and turnover. The retardation rate was a reflection of the slow mineralization and slow downward transport rates of organic-Pb complexes, due to sorption and desorption reactions involving mineral surfaces.

In a study involving stable Pb isotopes, Bindler et al. (2008) showed that Pb with a different isotopic composition could be detected in the soil down to a depth of at least 30 cm and sometimes down to 80 cm in Swedish soils. In comparison, in North American podzols, pollution Pb is typically only identified to a depth of 10-20 cm (even with the aid of isotopes). This difference is attributed to the longer history of metal pollution in Europe (as has been traced using lake sediments).

Several research groups have attempted to determine the mean residence time of Pb in the O-horizon of forest soils. Klaminder et al. (2006a) used three independent methods to estimate a mean residence time of about 250 years for Pb in the O-horizon of boreal forests in Sweden, indicating that deposited atmospheric Pb pollution is stored in the near-surface layers for a considerable period and, consequently, will respond only slowly to the reduction in atmospheric inputs. It should be noted, however, the OM in the upper parts of the O-horizon is continually being replaced by fresh litter and the mean residence time of Pb in these horizons is only 1-2 years. Thus, the uppermost layer will respond more quickly than the rest of the O-horizon to the decreases in Pb inputs.

Klaminder et al. (2008a) considered the biogeochemical behavior of atmospherically derived Pb in boreal forest soils in Sweden (Figure 2-11). The estimated annual losses via percolating soil water were ~2 mg/m^2 per year (Klaminder et al., 2008a) and so the annual loss, assumed to be from the mor layer, the well-defined layer of acidic humus that forms within the O-horizon in cool and moist soils, was greater than the atmospheric input of ~0.5 mg/m^2 per year. The upward transport of Pb did not compensate for the losses either. In contrast, the amount of Pb being stored in the mineral soil layers was increasing. The mean residence time of Pb in the mor layer was estimated to be ~300 years, in reasonable agreement with their earlier work (Klaminder et al., 2006a). These values were greater than the values of 2-150 years determined for U.S. forest soils, e.g., (Watmough et al., 2004; Kaste et al., 2003) but the difference was attributed to the lower decomposition rates of OM within the northern boreal forests of Sweden. They concluded that more research was needed to determine the processes occurring within the mor layer that control the release of Pb from this horizon.

Klaminder et al. (2008b) investigated in more detail the distribution and isotopic signature of Pb persisting within the O-horizon (mor layer) of boreal forest soils. They found that the mor layer preserved a record of past Pb emissions from a nearby smelter. Minimal animal burrowing activity and low leaching rates observed at the sampling location were important factors contributing to the preservation of this record. They concluded that temporal changes in atmospheric fallout in addition to adsorption processes need to be considered when interpreting Pb concentration changes within the mor layer.



Notes: The atmospheric deposition rate is from (Klaminder et al., 2006a), the plant uptake rates from (Klaminder et al., 2005) and estimated soil-water fluxes from (Klaminder et al., 2006b).

Figure 2-11 Schematic model summarizing the estimated flux of Pb within a typical podzol profile from northern Sweden using data from Klaminder et al. (2006a) and Klaminder et al. (2006b).

Significantly higher O-horizon Pb concentrations have been observed in coniferous than deciduous forest soils (McGee et al., 2007). Steinnes et al. (2005) noted evidence for downward migration of Pb from the O-horizon to the E-horizon of most soils and in some cases the upper B-horizon. They found that the downward transport of Pb differed considerably between the sites, e.g., from almost no anthropogenic Pb in the B-horizon at some sites to ~70% at other sites. The greater downwards transport in some locations was attributed to climatic variations, with more extensive leaching and possibly a greater turnover of OM at sites where higher mean annual temperatures were experienced. Higher atmospheric deposition of acidifying substances in these locations was considered the most important factor in Pb transport, causing release of Pb from exchange sites in the humus layer and promoting downward leaching.

Seasonal variation in Pb mobility has also been observed in forest soil. Other research indicated that Pb concentrations correlated with DOC concentrations in the soil solution from the O-horizon, and were lower during late winter and spring compared with summer months (Landre et al., 2009). The degradation of OM in the O-horizon produced high DOC concentrations in the soil solution. It was also shown that Pb was associated with the DOC, and concluded that DOC production is a primary factor enhancing metal mobility in this horizon. In the underlying mineral horizons, DOC concentrations declined due to adsorption and cation exchange processes. The B-horizon retained most of the DOC leached from the O-horizon and it has also been observed that Pb is similarly retained.

Non-forested Soils

In contrast with forest soils, most non-forested soils are less acidic and so most studies of Pb behavior in non-forested soils have focused on Pb immobility. However, there are acid soils in some locations that are not forested. For these soils, as for forest soils, Pb mobility is weak but correlated with OM. For example, Schwab et al. (2008) observed that low molecular weight organic acids added to soil enhanced Pb movement only slightly. Citric acid and tartaric acid enhanced Pb transport to the greatest degree but the extent of mobilization was only slightly higher than that attained using deionized water even at high concentrations. While the formation of stable solution complexes and more acidic conditions favored mobilization of Zn and Cd, Pb remained strongly sorbed to soil particles and so the presence of complexing agents and low pH (2.8-3.8) did not substantially enhance Pb mobility. Similarly, limited penetration and leaching was observed in an extremely complex temperate soil profile, with highest concentrations of Pb (~80 mg/kg) found in the top 0-5 cm section of soil. For this uppermost soil section, there was a strong correlation between Pb concentration and OC content, both for the total soil fraction and the acid-extractable fraction. The Pb migration rate was calculated to be 0.01 cm/year. It was estimated that Pb would be retained in the soil column for 20,000 years, with no evidence of rapid movement of anthropogenic Pb from the top 0-5 cm soil section into the soil profile Kylander et al. (2008).

Other recent studies also reported strong retention in non-forest soils and enhanced mobility of Fe and OM colloids. Pb was strongly retained on acidic Mediterranean soil columns, and association of Pb with the exchangeable OM and crystalline Fe oxide fractions appeared to favor mobility, while Pb association with Mn oxides and amorphous Fe oxides was linked with semi-irreversible retention of Pb in the solid phase (Garrido et al., 2008). In another study of Pb mobility within Mediterranean soils, Pb infiltration velocity was measured to be 0.005 meter/year (Erel, 1998). The authors attributed Pb movement within the soil column to advection and concluded that the soil

profile of Pb is similar to the anthropogenic air Pb emissions trend. Pedrot et al. (2008) studied colloid-mediated trace element release at the soil/water interface and showed that Pb was mobilized by Fe nanoparticles that were bound to humic acids.

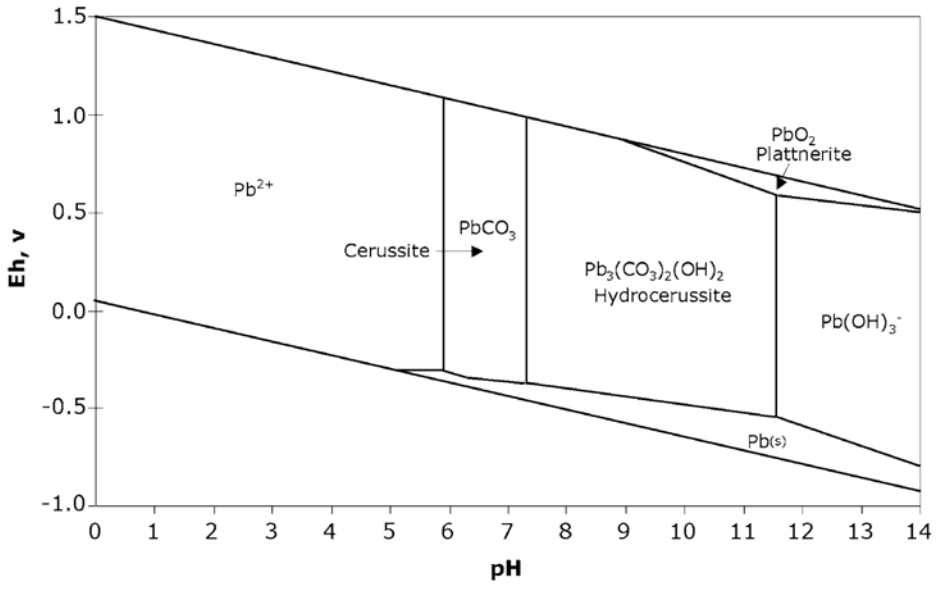
Soil pH value is probably the single most important factor affecting solubility, mobility and phytoavailability but reducing conditions also results in increased Pb mobility, with the release of Pb into an anoxic soil solution due to the combined effect of Fe(III) reductive dissolution and dissolved OM release. Dissolved OM is more important than Fe oxyhydroxides in determining Pb mobility. Under oxic conditions, Fe-Mn-hydroxides often play an important role in the sorption of Pb to the solid phase soil (Schulz-Zunkel and Krueger, 2009). In an agricultural soil, fate of Pb in soils is related to agricultural management. Although Pb was found to be strongly sorbed to the soil, downward migration was observed and the movement of Pb to deeper soils was due to the soil mixing activities of earthworms (Fernandez et al., 2007). Thus in relatively unpolluted non-forested soils, as in forested soils, colloidal Fe and OM, pH, and biophysical transport all enhance Pb mobility in soil. Pb transport in more highly contaminated soils has also been the subject of recent research. In a vegetated roadside soil, Pb was leached from the upper 50 cm of the soil even though the pH was 7.2. Pb was transported on mobile particles and colloids in the soil solution. Some of the colloids may have formed from OM produced by roots and decaying shoots. The transport process was enhanced by preferential flow triggered by intense rainfall events. This study suggested that the value of the effective sorption coefficient estimated under dynamic conditions was unrelated to values measured in conventional batch studies. This indicates that the use of batch studies to derive input values for sorption coefficients in transport models requires caution. It was concluded that the primary control of Pb transport in the long term was the degree of preferential flow in the system (Roulier et al., 2008b).

Other studies also noted similarly low Pb mobility, but with substantial variation between soil types and locations. A decline in O-horizon Pb concentrations and Pb accumulation in mineral horizons was also observed for forest soils by Watmough and Dillon (2007), but did not hold for nearby wetland areas from which a large amount of DOC is exported, with approximately 10 times more Pb being associated with a given amount of DOC in the leachate from the LFH-horizon of the wetland soil than with the DOC in the stream water draining the wetland. This may reflect greater retention of Pb by the wetland and/or a change in structure of DOC leading to a change in complexing capacity possibly because of changes in pH or competition with Al and Fe.

Williams et al. (2006) characterized Pb speciation in a mine waste-derived fertilizer, ironite. It was thought that PbS would be the main form of Pb, but instead was the predominant form was $PbSO_4$, which may move more easily through soil and enter

proximal waters. In contrast, Courtin-Nomade et al. (2008) showed that Pb was incorporated into barite rather than goethite in waste rock pile materials. The high-stability phase formed was an anglesite-barite solid solution.

In weathering flotation residues of a Zn-Pb sulfide mine, more Pb was mobile in weathered topsoil than in the unweathered subsoil (Schuwirth et al., 2007). The topsoil had a very high OM content and the Pb enrichment was attributed to an interaction with soil OM. Overall, the results contrast strongly with most other studies but the interpretation was supported by the sequential extraction results which showed that there was a very large exchangeable Pb component in these surface soils. Scheetz and Rimstidt (2009) characterized shooting range soils in Jefferson National Forest, VA, in which the metallic Pb shot rapidly became corroded and developed a coating of hydrocerussite, which dissolved at the pH values of 8-9; see Figure 2-12, which shows an Eh-pH diagram indicating the solubility, equilibrium, and stability of these corroded Pb molecules in terms of the activity of hydrogen ions (pH) versus the activity of electrons (Eh [in volts]). The solubilized Pb was largely re-adsorbed by the Fe and Mn oxides and carbonate soil fractions. The minimum solubility of hydrocerussite lies in the pH range 8-9 but solubility increases by several orders of magnitude at pH below 6 (Scheetz and Rimstidt, 2009).



Source: Reprinted with permission of Elsevier Publishing, Scheetz and Rimstidt (2009)

Figure 2-12 Eh-pH diagram for Pb in shooting range soils, Jefferson National Forest, VA.

Rooney et al. (2007) also investigated the controls on Pb solubility in soils contaminated with Pb shot. Again, corrosion crusts were found to develop on Pb pellets. The concentrations of Pb in the soil solution were, however, much lower than if they were controlled by the solubility of the dominant crustal Pb compounds (mainly hydrocerussite). Instead it was suggested that the concentrations were being controlled by sorption of Pb by the soil solid phase. The pH range in this study was 4.5-6.5 and so again dissolution of hydrocerussite would be expected. Sorption to solid phases in the soil is also consistent with the findings of Scheetz and Rimstidt (2009). Overall, in contrast to less polluted forested and non-forested soils, considerable mobility was often, but not always observed in soils near roadways and mines and on shooting ranges, with colloid transport and soil pH playing an important role in Pb mobility. Although there have been steep declines in Pb deposition, surface soils in have been slow to recover (Bindler et al., 2008; Kaste et al., 2006). As was concluded in the 2006 Pb AQCD (U.S. EPA, 2006b), soils continue to act as a predominant sink for Pb.

While in some studies the flux of Pb, from the soil through aquatic ecosystems to lakes has peaked and declined. In other studies, no recovery of lake sediments in response to emission reductions was observed (Norton, 2007). For example, Klaminder et al. (2010) has shown that the Pb concentrations in sub-Arctic lake sediments remain unchanged in recent years, with the lack of recovery linked to the effects of soil warming, which affect Pb-OM transport from soil to the receiving lake systems. Shotyk and Krachler (2010) also reported a disconnect between atmospheric deposition and recent changes in Pb concentration and isotope ratios in the lake sediments. Simulations of future metal behavior suggest that the more strongly sorbing metals such as Pb will respond to changes in metal inputs or acidification status only over centuries to millennia (Tipping et al., 2006).

Overall, recent research confirms the generally low mobility of Pb in soil. This limited mobility is strongly dependent on both colloid amount and composition, as well as pH, and may be greater in some contaminated soils. Mobility is so low that soils continue to act as a sink for atmospheric Pb even though atmospheric Pb concentrations peaked several decades ago.

2.4 Monitoring of Ambient Pb

2.4.1 Measurement Techniques

2.4.1.1 Sample Collection

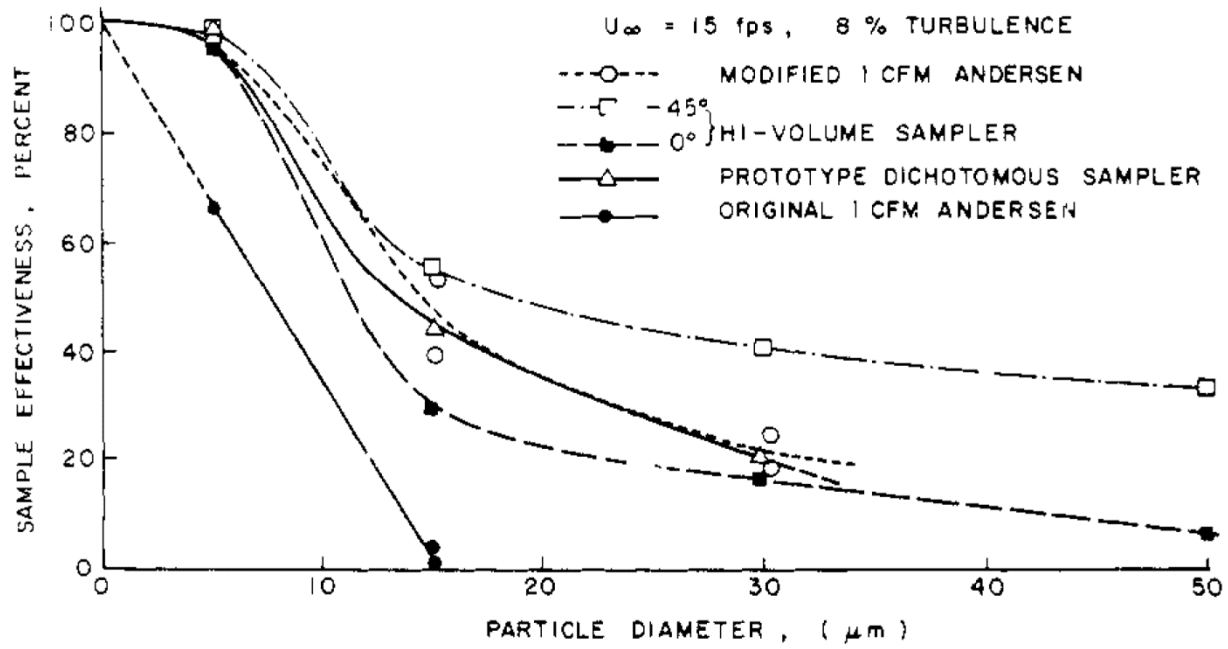
Federal Reference Methods

The indicator for the Pb NAAQS is Pb in total suspended particles (Pb-TSP) (73 FR 66964). In order to be used in regulatory decisions judging attainment of the Pb NAAQS, ambient Pb concentration data must be obtained for this indicator using either the Federal Reference Method (FRM) or a Federal Equivalent Method (FEM) defined for this purpose. Accordingly, for enforcement of the air quality standards set forth under the Clean Air Act, EPA has established provisions in the Code of Federal Regulations under which analytical methods can be designated as FRM or FEM. Measurements for determinations of NAAQS compliance must be made with FRMs or FEMs. FRMs and FEMs for the Pb NAAQS exist for both sample collection and sample analysis.

There are two FRMs for sample collection in the Pb NAAQS monitoring network (described in Section 2.4.2): (1) Reference Method for the Determination of Lead in Suspended Particulate Matter Collected From Ambient Air (40 CFR part 50 Appendix G), and (2) Reference Method for the Determination of Lead in Particulate Matter as PM_{10} Collected From Ambient Air (40 CFR part 50, Appendix G). The Pb-TSP FRM sample collection method is required for all source-oriented NAAQS monitors, and the FRM for Pb-PM_{10} is accepted for Pb NAAQS monitoring at non-source-oriented monitors in specified situations.

The Pb-TSP FRM sample collection method specifies use of a high-volume TSP sampler that meets specified design criteria (40 CFR part 50 Appendix B). Ambient airborne PM is collected on a glass fiber filter for 24 hours using a high volume air sampler. It has long been recognized that there is notable variability in high-volume TSP sample measurements associated with the effects of wind speed and wind direction on collection efficiency. This variability is predominantly associated with the capture efficiency for particles larger than 10 µm, but the sampler's size selective performance is known to be affected by wind speed and direction. For example, at a simulated wind speed of

4.6 meters/second, a directional difference of 45 degrees can result in a nearly two-fold difference in 15 µm particle collection efficiency and a nearly five-fold difference in 50 µm particle collection efficiency (Wedding et al., 1977). Effective D_{50} (size at 50% efficiency) was observed to decrease from 50 µm at a 2 km/hour wind speed to 22 µm at 24 km/hour (Rodes and Evans, 1985). Figure 2-13 illustrates the effect of sampler orientation on collection efficiency as a function of particle size.



Different TSP sampler types: (1) Modified Andersen Sampler [open circles]; (2) Hi-volume Sampler (for different incident wind direction (45° [open squares], 0° [closed squares]); (3) Prototype 15 µm Cutpoint Dichotomous Sampler [open triangles]; and (4) Original Andersen Sampler [closed circles].

Source: Reprinted with permission of the American Chemical Society (Wedding et al., 1977)

Figure 2-13 Comparison of particle collection efficiency among different TSP sampler types.

Some existing commercially available sampler inlets are designed to collect particles larger than 10 µm with greater than 50% efficiency (Kenny et al., 2005), and these inlets can be tested as potential replacements for TSP sampling. Efficient collection of particles much larger than 10 µm is considerably more challenging because their greater inertia and higher settling velocities hinder their efficient intake by samplers. The sampling difficulties and the long history of research to develop adequate sampling technology for large particles have been thoroughly reviewed (Garland and Nicholson, 1991). High intake velocities and large inlet openings are necessary to minimize sampling bias for

sampling ultra-coarse particles. At this time, no alternative to the FRM TSP sampler has been identified that has been adequately characterized. As such, there is a continued need to assess the feasibility of a revised TSP sampler design with improved control on collection efficiency over a wider range of particle sizes, including ultra-coarse particles (which are not captured with PM_{10} samplers).

The spatial scale for which ambient air Pb samples are representative varies depending on particle sizes present, as discussed further in Section 2.5.3. Concentrations of particles larger than 10 µm are likely to be very spatially and temporally heterogeneous, with higher concentrations in the vicinity of their emissions sources. Under typical conditions, $PM_{10-2.5}$ particles travel much shorter distances before settling out than finer particles (U.S. EPA, 2009a). As a result, spatial and temporal heterogeneity is greater for $PM_{10-2.5}$ than for $PM_{2.5}$, because coarser particles have greater settling velocities (Hinds, 1999), and settling velocities are even greater for particles larger than 10 µm. Thus, spatial gradients are steepest near sources, such that measured concentrations of larger particle sizes tend to be most representative of the ambient air in areas in close proximity to the monitor, with higher concentrations likely to occur closer to the source and decreasing concentrations with increasing distance from the source. This issue has been thoroughly discussed in the 2006 Pb AQCD (U.S. EPA, 2006b). It has also been acknowledged in previous Pb AQCDs, with a lengthy discussion appearing in the 1977 AQCD (U.S. EPA, 1986b, 1977).

The low-volume Pb-PM_{10} FRM sample collection method specifies use of a low-volume PM_{10} sampler that meets specified design criteria (40 CFR part 50, Appendix Q). Ambient airborne PM is collected on a polytetrafluoroethylene (PTFE) filter for 24 hours using active sampling at local conditions with a low-volume PM_{10} sampler and analyzed by X-ray fluorescence (XRF). In recognition of the steep spatial gradients associated with sources of ultracoarse particles, ambient Pb sampled using the FRM for Pb-PM_{10} is allowed in certain instances where the expected Pb concentration does not approach the NAAQS and no sources of ultracoarse Pb are nearby.

Alternative Sample Collection Methods

In addition to the FRMs for ambient Pb sample collection, a range of other PM sampling methods are available for collecting samples for Pb analysis. These include FRM sampling methods for PM that have also been used for collection of samples for Pb analysis, sampling methods in use in other sampling networks such as the CSN, IMPROVE and National Air Toxics Trends Stations (NATTS) networks described in Section 2.4.2, and other sampling methods that have been used to measure airborne Pb concentrations in research studies unrelated to network applications; these methods are

listed in Table 2-3. Monitors that are not FRM sampling methods but are stated by their manufacturer to capture TSP are called “manufacturer-designated TSP inlets” (denoted in the text by “TSP”) for this discussion. Most of these methods have been described in considerable detail in the 2004 PM AQCD (U.S. EPA, 2004). Table 2-3 also lists key conditions of capture for each method, including particle size, inlet type, collection medium, and flow rate. Not all methods included in Table 2-3 and in subsequent paragraphs have been applied to Pb-PM collection, but these methods represent potential alternative methods for this purpose. It should also be noted that not all of the samplers listed in Table 2-3 have been wind tunnel tested for a variety of aerodynamic particle sizes, wind speeds, and wind directions. In addition, some of these samplers are no longer commercially available.

Size discrimination is usually accomplished with impactors or cyclones. With impactors, PM is forced through a jet at high speed, and particle inertia carries particles above a given size into a collection surface downstream of the jet, while smaller particles follow the air stream around the collector. In multistage impactors, a series of successive stages of jets are used to collect a range of particle sizes. The micro-orifice uniform deposit impactor (MOUDI) is a widely used multistage impactor. The impaction process and performance of various impactors, including the WINS and MOUDI, has been described in detail in the 2004 PM AQCD (U.S. EPA, 2004). The biggest concern in collection by impaction is particle bounce, which occurs when particles collide with the collection surface but bounce off the collection stage into the air stream and are not actually collected. Considerable effort has been devoted to minimizing errors due to bounce in FRM samplers, and this has been thoroughly discussed in the 2004 PM AQCD (U.S. EPA, 2004). An alternative to impaction that also eliminates particle bounce is the use of an air sampling cyclone. In the CSN and IMPROVE networks, cyclones are used to remove particles larger than 2.5 μm. An air sampling cyclone brings air into a tangential jet and directs flow against a circular wall, where particles larger than a given size are removed by centrifugal and gravitational forces.

Table 2-3 Airborne PM sampling methods potentially applicable for Pb sampling.

Sampler	Network	Sampler Type	Particle Size Characteristics	Inlet or Fractionator Type	Collection Medium	Typical Flow Rate	Reference
High Volume TSP	Pb-FRM	Single Channel	TSP	Gabled, rectangular	Glass	1130 L/min	U.S. EPA (2011f)
Low Volume PM_{10}	PM-FRM, NATTS	Single Channel	MMAD <10 µm	Louvered Inlet + PM_{10} Impactor	Teflon	16.67 L/min	U.S. EPA (2011f)
$PM_{2.5}$	PM-FRM	Single Channel	MMAD <2.5 µm	WINS Impactor or (VSCC)	Teflon	16.67 L/min	U.S. EPA (2011f)
Met One SASS	CSN	Multiple Channel	MMAD <2.5 µm	Cyclone	Teflon	6.7 L/min	MetOne (2009)
IMPROVE	IMPROVE	Multiple Channel	MMAD <2.5 µm	Cyclone	Teflon	22.8 L/min	CNL (2001)
MOUDI	None	Multistage Impactor	8 stages MMAD 0.056-18 µm	Impactor	Teflon	30 L/min	Marple et al. (1991)
Noll Impactor	None	Multistage Impactor	4 stages MMAD <108 µm	Impactor	Coated Mylar	Rotating arm	Noll (1970)
SEAS	None	Slurry	MMAD <1.2 µm	Impactor	Slurry	90 L/min	Pancras et al. (2006), Ondov et al. (2006)
PM_{10} SSI HiVol	FRM	Single Channel	MMAD <10 µm	PM_{10} Size Selective Inlet	8” x 10” filter paper	1130 L/min	U.S. EPA (2011f)
Andersen Inhalable PM Sampler	FRM	Single Channel	MMAD <10 µm	RAAS10 Inlet	Teflon	16.67 L/min	U.S. EPA (2011f)
Sierra Dichotomous Sampler	FRM	Dichotomous	MMAD <10 µm, MMAD <2.5 µm	10 µm Inlet + Virtual Impactor	Teflon	16.7 L/min	U.S. EPA (2011f)
TEOM	FRM	Single Channel	MMAD <10 µm	R&P PM_{10} Inlet or Louvered Inlet	Teflon Coated Glass Fiber	16.7 L/min	U.S. EPA (2011f)

Table 2-3 (Continued): Airborne PM sampling methods potentially applicable for Pb sampling.

Sampler	Network	Sampler Type	Particle Size Characteristics	Inlet or Fractionator Type	Collection Medium	Typical Flow Rate	Reference
Louvered Inlet "TSP"[a]	None	Single Channel	MMAD <15 µm	Louvered Inlet	Teflon	16.67 L/min	Kenny et al. (2005)
Texas A&M Lo-Vol "TSP"[a]	None	Single Channel	"TSP"	None	Teflon	16.67 L/min	Wang et al. (2005b); Wanjura et al. (2005)
Andersen Multistage Impactor	None	Multistage Impactor	8 stages MMAD 0.4-10 µm and above	Inlet Cone	Aluminum	28.3 L/min	Mercer et al. (1970)
UIUC Isokinetic "TSP"[a] Sampler	None	Single Channel	"TSP"	Isokinetic Sampling Head	Teflon	20 L/min	Jerez et al. (2006)
Airmetric MiniVol	None	Single Channel	"TSP" or MMAD <10 µm or MMAD <2.5 µm	None, PM_{10} Impactor, or $PM_{2.5}$ Impactor	Teflon	5 L/min	Chen et al. (2011a)
Stacked Filter Units	None	Two stage impactor	MMAD <10 µm, MMAD <2.5 µm	PM_{10} Size Selective Inlet	Nuclepore	17 L/min	IAEA (1993)
ELPI	None	Multistage Impactor	13 stages MMAD 0.007-10 µm	Berner Impactor	Aluminum or polycarbonate	10 or 30 L/min	Keskinen et al. (1992)
Wagner & Leith Passive Sampler	None	Passive	Not characterized	Mesh screen	SEM stub	0 L/min	Leith et al. (2007); Wagner and Leith (2001)

[a] Monitors that are not FRM sampling methods but are stated by their manufacturer to capture TSP are called "manufacturer-designated TSP inlets" (denoted by "TSP").

Collection medium and flow rate are two other key features of a sampling method. One advantage of low volume sampling is its suitability for collection of samples for XRF analysis. Because Pb in $PM_{2.5}$ is analyzed by XRF in the CSN and IMPROVE networks, sampling methods that employ Teflon filters suitable for XRF analysis have been developed for these networks. In practice, this restricts sampling for airborne Pb to low volume samplers with a convenient filter size. This also holds true for the Pb-PM_{10} FRM sampling, which is also restricted to low volume PM_{10} samplers because XRF has been designated as the FRM for Pb-PM_{10} analysis. An additional practical advantage of available low volume samplers over the existing high volume Pb-TSP FRM is that established low volume $PM_{2.5}$ and PM_{10} sampling methods are not dependent on wind direction. However, this has to do with sampler design rather than flow rate, and there are high volume PM_{10} sampling methods, including the PM_{10} FRMs, that are also free of wind direction bias. These would be suitable for Pb measurement with other analytical methods, such as ICPMS, and could have a potential advantage of providing more material in locations with very low concentrations.

Alternatives to TSP sampling have been developed that collect a particle size range that extends beyond 10 μm. Early examples include samplers for "inhalable particulate matter" that were designed to have cut points for 50% sampling efficiency of 15 μm aerodynamic diameter. These included the Andersen Inhalable Particulate Sampler (Model 7000, Thermo Electron, Smyrna, GA) and the Sierra Dichotomous Sampler (Series 244, Sierra Instruments, Monterey, CA), which were evaluated and compared to each other and to TSP sampling in both co-located field comparisons (Solomon et al., 1982) and wind tunnel studies (Watson et al., 1983), with the result that poor agreement was observed for low or high wind speeds, or when much coarse particulate matter was present. For example, the Dichotomous Sampler collected on average only 73 ± 18% as much mass as the Inhalable Particulate Sampler, and differences were attributed to differences in the efficiency of large particle collection (Solomon et al., 1982).

More recently, a variety of inlets have been developed for low volume "TSP" sampling. The omnidirectional TEOM "TSP" Inlet (Model 10-002929, Rupprecht & Pataschnik Co., Inc, Albany, NY) was designed to sample 100 μm particles in still air with the suction velocity equal to the terminal velocity of a 100 μm diameter unit density sphere. However, substantial PM mass loss was reported for this inlet and attributed to anisokinetic sampling conditions that led to inefficient sampling of large particles (Jerez et al., 2006). The inefficient sampling of larger particles by the "TSP" inlet was also observed by Kenny et al. (2005), who carried out wind tunnel tests of 1) a commercially available omnidirectional low volume (16.67 liters/minute) "TSP" inlet, and 2) a louvered dichotomous inlet designed to select particles from a moving airstream and transmit them to a downstream PM_{10} impactor. They observed that the "TSP" inlet

exhibited low sampling efficiency even for larger particles within the PM_{10} range and concluded that it is likely to give biased PM concentrations that vary with external winds when large particles are present. However, for the louvered dichotomous inlet, Kenny et al. (2005) reported high sampling efficiencies with little influence of wind speed across the full PM_{10} particle size range, and a 50% cutpoint at around 15 μm. For the full scale "TSP," inlet sampling efficiencies for 46 μm particles were 17% for a 1 meter/second wind speed and 28% at 2 meters/second.

Evaluation of other available "TSP" samplers also reveals variability among sampler models. Jerez et al. (2006) compared the University of Illinois at Urbana Champaign (UIUC) isokinetic "TSP" sampler with a tapered element oscillating microbalance (TEOM) "TSP" sampler (Series 1400a, Rupprecht and Pataschnik Co., Inc., Albany, NY) when measuring dust concentrations in swine and chicken houses in Illinois, Indiana, Minnesota, and Texas between September to December, 2003. The TEOM measured concentrations that were 26-117% of the UIUC sampler; for 86 of 90 measurements, the TEOM measurements were lower than the UIUC measurements.

Other new approaches to high volume sampling include "saturation samplers" or low volume (5 liters/minute) samplers designed for high spatial coverage of PM_{10} and $PM_{2.5}$. Chen et al. (2011a) provide an intercomparison among portable MiniVol (Airmetric, Eugene, OR), Omni (BGI, Inc., Waltham, MA), and dichotomous samplers (Model 2025, Rupprecht and Pataschnik, Albany, NY) and two FRMs (RAAS-100, Andersen, Smyrna, GA; Partisol-FRM 2000, Rupprecht and Pataschnik, Albany, NY) for PM_{10} and $PM_{2.5}$ measurements. Chen et al. (2011a) observed R^2 of 0.95-0.98 with average mass concentrations within 4% among the PM_{10} measurements. They observed R^2 of 0.96-0.99 and average mass concentrations within 9% among the monitors for $PM_{2.5}$ measurements. Hitzenberger et al. (2004) found more variability among portable $PM_{2.5}$ and PM_{10} monitors when performing intercomparisons in Melpitz, Germany. $PM_{2.5}$ was measured with stacked filter units (SFU) (developed at Ghent University), a TEOM (Model 1400A, Rupprecht and Pataschnick, Albany, NY), a Digitel (Model DHA-80) high volume sampler, an electrical low pressure impactor (ELPI) (Outdoor-ELPI, Dekati, Ltd.), a TSP, and a MOUDI. PM_{10} was measured with SFUs, a "TSP" (manufacturer not provided) with 30 L/min and 70 L/min Berner impactors, and a MOUDI (model and manufacturer not provided). Based on Hitzenberger et al.'s (2004) reported average mass concentrations, the $PM_{2.5}$ samplers ranged from 73% (TEOM) to 180% (ELPI) of the average. When trimming the extrema, the average $PM_{2.5}$ concentrations measured within 10% of the overall average. For the PM_{10} monitors, Hitzenberger et al.'s (2004) reported average mass concentrations ranged from 83% (one SFU, MOUDI) to 123% (two SFUs) of the overall average. When trimming the extrema, the average PM_{10} concentrations were within 13%.

Passive samplers have also recently been used for capturing spatial variability of ambient air Pb concentrations. Field testing of the Wagner and Leith Passive Sampler (University of North Carolina) illustrated very good agreement with a cascade impactor (difference within 5% for three sampling events) for $PM_{2.5}$ in certain cases and very poor agreement (difference of -51% and -110%) in others; Wagner and Leith (2001) attributed the poor agreement to low $PM_{2.5}$ mass events. PM_{10} agreement was 14-65% and was attributed to potential phenomena such as agglomeration on the passive sampler and water evaporation from the impactor sample. However, when comparing the Wagner and Leith Passive Sampler for $PM_{10-2.5}$ with $PM_{10-2.5}$ calculated by differencing measurements obtained from co-located or dichotomous FRMs for PM_{10} and $PM_{2.5}$, Leith et al. (2007) observed that Passive Sampler $PM_{10-2.5}$ measurements integrated over at least one week were within one standard deviation of the $PM_{10-2.5}$ obtained from co-located or dichotomous FRMs. Kumar et al. (2012) and Lagudu et al. (2011) illustrated how the Wagner and Leith Passive Sampler can be coupled with computer-controlled scanning electron microscopy (CCSEM) to produce concentrations of Pb-$PM_{10-2.5}$ in samples taken across the cities of Syracuse, NY and Rochester, NY, respectively.

These results illustrate that alternative sampling options to TSP are available to capture ambient air Pb concentrations for particles with a cutpoint of approximately 15 μm or higher, and the state of the science for sampling in this particle size range is progressing, including better documentation of performance and field intercomparison data. In general, both the historical and recently available alternatives to the traditional TSP sampler illustrate that samplers designed to collect particles up to a size range greater than 10 μm have not performed or compared as well as samplers designed to collect smaller particles, and the challenge of achieving good performance for collection of particle size ranges greater than 10 μm has not been limited to the TSP sampler. This is expected given the inherent difficulties associated with large particle sampling (Garland and Nicholson, 1991). In spite of this, good performance by recently developed samplers, including the louvered “TSP” inlet evaluated by Kenny et al. (2005) show promise for good performance in collection of a particle size range extending beyond 10 μm. The primary route of Pb exposure is hand-to-mouth contact with deposited Pb on soil or dust having substantially larger size fractions compared with airborne Pb particles, as described in Section 3.1. However, the relevant particle size distribution for ambient sampling is smaller than the size distribution of the settled dust, as described in Section 3.1.1.1. Particles larger than about 20 μm are generally considered too large to be transported for more than a few seconds under typical conditions; see Section 2.3.1.3. If preliminary results concerning the “TSP” louvered inlet and other “TSP” alternatives are verified, this may be very close to the practical limit for good sampling data quality. It

follows that a size cut-off around 15 μm may achieve both good data quality and representative sampling in a limited area.

2.4.1.2 Sample Analysis: Federal Reference and Federal Equivalence Methods

As described in Section 2.4.1.1, measurements for determinations of NAAQS compliance must be made with FRMs or FEMs. As of October 12, 2011, one manual reference method and 25 manual equivalent methods for sample analysis had been approved for Pb (http://www.epa.gov/ttn/amtic/files/ambient/criteria/reference-equivalent-methods-list.pdf). The FRM for Pb (Pb-TSP) was promulgated in 1979 and is based on flame atomic absorption spectroscopy (AAS) (40 CFR Part 50, Appendix G). Ambient air suspended in PM is collected on a glass fiber filter for 24 hours using a high volume air sampler. Pb in PM is then solubilized by extraction with nitric acid (HNO_3), facilitated by heat, or by a mixture of HNO_3 and hydrochloric acid (HCl) facilitated by ultrasonication. The Pb content of the sample is analyzed by atomic absorption spectrometry using an air-acetylene flame, using the 283.3 or 217.0 nm Pb absorption line, and the optimum instrumental conditions recommended by the manufacturer. Several FEMs have been approved based on a variety of principles of operation have been approved, including inductively coupled plasma optical emission spectrometry, and inductively-coupled plasma mass spectrometry (ICPMS).

Atomic Absorption Spectrometry

AAS is the basis for the existing FRM. Atomic absorption spectrometry was first developed in the 19th century, and became widely used in the 1950s. More than 70 elements can be analyzed by AAS. Typically a liquid sample is nebulized into a flame with sufficient heat for elements to be atomized. The liquid specified by the FRM is a nitric acid extract of a glass fiber filter used for collection of suspended PM with a high volume sampler. The atomized sample is then irradiated with visible light at a specific wavelength to promote an electronic transition to a short-lived excited state, resulting in absorption of the light. Elemental selectivity is achieved because light absorption is specific to a particular electronic transition in a particular element. As a result, absorption of light at a given wavelength generally corresponds to only one element. The flame is irradiated with a known quantity of light and intensity of light is measured on the other side of the flame to determine the extent of light absorption in the flame. Using the Beer-Lambert law the concentration of the element is determined from the decrease in light intensity due to sample absorption.

A more sensitive variation of atomic absorption spectrometry for most elements is graphite furnace atomic absorption spectrometry (GFAAS). Instead of introducing the sample into a flame, the liquid sample is deposited in a graphite tube that is then heated to vaporize and atomize the sample.

Inductively-Coupled Plasma Mass Spectrometry

Inductively coupled plasma mass spectrometry (ICPMS) is a sensitive method of elemental analysis developed in the late 1980s. Argon (Ar) plasma (ionized gas) is produced by transmitting radio frequency electromagnetic radiation into hot argon gas with a coupling coil. Temperatures on the order of 10,000 K are achieved, which is sufficient for ionization of elements. Liquid samples can be introduced into the plasma by extracting samples in an acid solution or water and nebulizing dissolved elements. Resulting ions are then separated by their mass to charge ratio with a quadrupole and signals are quantified by comparison to calibration standards. While solid samples can be introduced by laser ablation, nebulization of liquid extracts of PM collected on Teflon filters is more typical. One major advantage of ICPMS over AAS is the ability to analyze a suite of elements simultaneously. An additional advantage is low detection limits of 50-100 parts/trillion for Pb.

Inductively-Coupled Atomic Emission Spectroscopy

Inductively coupled atomic emission spectroscopy (ICP-AES) also generates ions from elements with a hot Ar plasma, similar to ICPMS. Excited atoms and ions are produced, and these emit electromagnetic radiation with frequencies characteristic of a particular element. Intensity of emission is used to determine the concentration of an element in the sample. Elements are extracted from filter samples and nebulized into the plasma.

Energy Dispersive X-ray Fluorescence

In energy dispersive X-ray fluorescence spectrometry a beam of X-ray photons from an external excitation source is applied to a sample, causing ejection of inner shell electrons from elements in the sample. Because inner shell electrons have higher electron binding energies than outer shell electrons, the ejection of the inner shell electron induces an energetically favorable electronic transition of an outer shell electron to replace the ejected electron. The energy released as a result of this transition is in the form of electromagnetic radiation, corresponding to the difference in electronic binding energies before and after the transition. The energy released is typically in the X-ray portion of the electromagnetic spectrum. The release of electromagnetic radiation as a result of an

electronic transition is defined as fluorescence. Fluorescence energies associated with electronic transitions depend on atomic structure, and vary between elements. As a result, X-ray fluorescence energy is uniquely characteristic of an element, and X-ray intensity at a given energy provides a quantitative measurement of elemental concentration in the sample. The X-rays are detected by passing them through a semiconductor material, resulting in an electrical current that depends on the energy of the X-ray.

2.4.1.3 Other Analysis Methods for Total Pb

Several other methods that have not been designated as FRM or FEM methods have also been frequently used to obtain atmospheric Pb measurements. These include proton induced x-ray emission (PIXE), X-ray photoelectron spectroscopy (XPS), and other methods

PIXE

Proton-induced X-ray emission (PIXE) spectroscopy has been widely used to measure Pb in atmospheric PM. In PIXE, a high-energy proton beam passes through the sample, causing electrons to be excited from inner shells. The x-rays emitted when electronic transition occur to replace the inner shell electrons are characteristic of an element and can be used to identify it. Development of PIXE for analysis of airborne PM was reviewed by Cahill et al. (1981). Numerous applications of PIXE to analysis of airborne Pb-PM have been reported in the past five years (Cohen et al., 2010; Waheed et al., 2010; Sanchez-Ccoyllo et al., 2009; Chan et al., 2008; Johnson et al., 2008; Cong et al., 2007; Ariola et al., 2006; Johnson et al., 2006; Wåhlin et al., 2006).

XPS

X-ray photoelectron spectroscopy (XPS), also called electron spectroscopy for chemical analysis (ESCA) has been used to determine Pb concentrations on materials surfaces, including atmospheric PM (Finlayson-Pitts and Pitts, 2000). A fixed frequency X-ray beam causes inner shell electrons to be emitted and kinetic energy of ejected electrons is measured. Binding energy characteristic of an element can be calculated from the measured kinetic energy, allowing identification of the element. XPS can also provide information about an element's chemical environment or oxidation states because of chemical shifts in binding energy caused by differences in chemical environment. There have been some recent applications of XPS to airborne PM, concluding that Pb was

mostly in the form of Pb sulfate (Qi et al., 2006). XPS analysis is a surface technique that is suitable only to a depth of 20-50Å.

Other Total Pb Methods

Anodic stripping voltammetry, atomic emission spectroscopy, and colorimetry have also been used for measurement of atmospheric Pb (Finlayson-Pitts and Pitts, 2000). In anodic stripping voltammetry, metal ions are reduced to metallic form and concentrated as an amalgam on a suitable electrode (e.g., a mercury (Hg) amalgam on a mercury (Hg) electrode). This is followed by re-oxidation in solution, which requires "stripping" the reduced metal from the electrode. Emission spectroscopy can be compared to the existing FRM for Pb based on AAS. In AAS, radiation absorbed by non-excited atoms in the vapor state is measured. In emission spectroscopy, radiation due to the transition of the electron back to ground state after absorption is measured, and the energy of the transition is used to uniquely identify an element in a sample. Colorimetric methods are wet chemical methods based on addition of reagents to a Pb containing solution to generate measurable light absorbing products. These methods are less sensitive than ICPMS, XRF, and PIXE and their use is declining as more sensitive methods become more widely used but have advantages regarding simplicity and cost.

2.4.1.4 Sequential Extraction

Sequential extraction has been widely used to further classify Pb for various purposes, including bioavailability, mobility, and chemical speciation. In general the more easily extractable Pb is considered more mobile in soil and is more bioavailable to organisms. This approach has also been used widely in characterization of airborne PM. In its original application (Tessier et al., 1979) metals extraction solvents were selected to correspond to common species present in soil, and metals were classified as exchangeable, bound to carbonates, bound to iron (Fe) and manganese (Mn) oxides, bound to OM, and residual. Extraction was carried out with successively stronger solutions, starting with magnesium chloride ($MgCl_2$) for removal of exchangeable metals and ending with hydrofluoric and perchloric acids for removal of residual metals. Pb was one of the elements originally studied by Tessier et al. (1979) as well as one the elements analyzed when Tessier's scheme was first applied to airborne PM (Fraser and Lum, 1983).

Tessier's scheme was modified and optimized for airborne PM over time (Fernandez Espinosa et al., 2002) and additional extraction schemes were also developed (Chester et al., 1989), including the simplest case of two fractions corresponding to soluble and

insoluble fractions (Falta et al., 2008; Canepari et al., 2006; Voutsa and Samara, 2002). The variety of methods in current use was recently thoroughly reviewed by Smichowski et al. (2005). With the recognition that biological processes involving deposited PM metals were related to their solubility (U.S. EPA, 2009a), sequential extraction methods or simpler schemes to divide metals into water and acid soluble fractions were increasingly applied to PM samples to obtain data not just on total metal concentration but also on water soluble concentration (Graney et al., 2004; Kyotani and Iwatsuki, 2002; Wang et al., 2002). Compared to other elements, a large fraction of total Pb is soluble (Graney et al., 2004). Recent advances in this area have included application to size fractionated PM (Dos Santos et al., 2009; Birmili et al., 2006), time resolved measurements (Perrino et al., 2010), and an extensive comparison of different fractionation schemes (Canepari et al., 2010). Sequential extraction with two or more fractions is becoming more widely used for characterization of Pb-PM in a variety of sources (Canepari et al., 2008; Smichowski et al., 2008; Poykio et al., 2007; Sillanpaa et al., 2005) and locations (Perrino et al., 2010; Dos Santos et al., 2009; Cizmecioglu and Muezzinoglu, 2008; Dahl et al., 2008; Sato et al., 2008; Annibaldi et al., 2007; Richter et al., 2007; Al-Masri et al., 2006; Canepari et al., 2006; Fujiwara et al., 2006; Wang et al., 2006c; Yadav and Rajamani, 2006; Gutierrez-Castillo et al., 2005; Heal et al., 2005), leading to a better understanding of mobility characteristics of Pb in airborne PM.

2.4.1.5 Speciation Techniques

XAFS

There have been few attempts to speciate Pb in atmospheric PM. However, recently X-ray absorption fine structure (XAFS) has been applied to PM and road dust to obtain Pb speciation data from direct analysis of particle surfaces. In XAFS the absolute position of the absorption edge can be used to determine the oxidation state of the absorbing atom, and scattering events that dominate in the near edge region provide data on vacant orbital energies, electronic configurations, and site symmetry of the absorbing atom that can be used to determine the geometry of the atoms surrounding the absorbing atom. XAFS can be divided into two spectral regions. X-ray absorption near edge structure (XANES) is the region of the x-ray absorption spectrum up to 50 eV above the absorption edge observed when an inner shell electron is electronically excited into unoccupied states, and Extended X-ray Absorption Fine Structure (EXAFS) up to 1 keV above the absorption edge. Both have been applied recently to Pb in PM. XANES spectra of Pb coordination complexes with a wide range of environmentally relevant ligands have been reported (Swarbrick et al., 2009). XANES has been used to show that several different Pb species

are probably present in urban airborne PM (Funasaka et al., 2008) and urban road dust (Barrett et al., 2010). XANES has been used to differentiate between Pb chromate, Pb-sorbed minerals, Pb chloride, Pb oxide, Pb carbonate, Pb sulfide and Pb sulfate, which are probably present in urban PM and road dust samples (Barrett et al., 2010; Funasaka et al., 2008; Tan et al., 2006). XANES has also been used to investigate Pb in air samples thought to be complexed with humic substances from soil (Pingitore et al., 2009) and to investigate the speciation of atmospheric Pb in soil after deposition (Guo et al., 2006b). EXAFS has been applied to emission sources to show Pb from a sinter plant was mainly carbonate (Sammut et al., 2010). XAFS has only been applied to airborne PM very recently and shows promise for chemical speciation of airborne metals, including Pb.

GC-ICPMS and HPLC-ICPMS

Environmental analytical methods for organolead compounds prior to 2000 were generally time consuming and costly, requiring extraction, derivatization, and detection (Quevauviller, 2000). These have been thoroughly reviewed (Pyrzyńska, 1996) and method intercomparison studies have been conducted (Quevauviller, 2000). More recently, speciation of organometallic compounds in environmental samples has usually been carried out by coupling a chromatographic separation step with a mass spectrometry-based multi-element detection system capable of analyzing a wide range of elements along with Pb, and these approaches have also been recently reviewed (Hirner, 2006). Chromatographic systems in common use are gas chromatography (GC) and high performance liquid chromatography (HPLC). Detection systems most commonly used are an inductively coupled plasma mass spectrometer (ICP-MS [ICPMS]), electron impact ionization mass spectrometry (EI-MS), and electrospray ionization mass spectrometry (ESI-MS) (Hirner, 2006). Using these techniques, organometallic species are separated from each other based on differences in retention times on chromatographic columns, and elemental Pb is determined by the ICPMS used as a detector downstream of the column to measure elemental Pb in the pure compounds after chromatographic separation. Pb speciation analysis has benefited from the development of HPLC-ICPMS in particular (Quevauviller, 2000). Recent advances in metal speciation analysis in environmental samples by HPLC-ICPMS have been extensively reviewed (Popp et al., 2010). HPLC-ICPMS has been used for analysis of Pb complexes with humic substances (Vogl and Heumann, 1997), which could be relevant for resuspended soil and road dust. GC-ICPMS has been more widely used for separation and analysis of methyl and ethyl Pb species in atmospheric PM (Poperechna and Heumann, 2005; Jitaru et al., 2004; Leal-Granadillo et al., 2000).

Pb-Isotope Ratio Analysis

Classifying Pb by its relative isotopic abundance has also proved useful for a variety of purposes, including the determination of its geochemical origins in natural samples and the relative contributions of coal burning, mining, smelting, and motor vehicle emissions in polluted samples (Farmer et al., 1996). Typically, isotopes of Pb (^{208}Pb, ^{207}Pb, ^{206}Pb, and ^{204}Pb) are measured in a sample using mass spectrometry, and then ratios of the isotopes are calculated to obtain a "signature." Isotopes of ^{208}Pb, ^{207}Pb, and ^{206}Pb are substantially more abundant than ^{204}Pb, but they vary depending on the geologic conditions under which the ore was produced through decay of different isotopes of uranium and thorium (Cheng and Hu, 2010). Isotope ratio analysis was first applied to airborne PM in 1965 to identify the impact of motor vehicle exhaust on marine and terrestrial Pb deposition in the Los Angeles area (Chow and Johnstone, 1965). More recently, high resolution ICPMS has also proved to be a sensitive tool for isotope ratio analysis. High resolution ICPMS was first applied to geological samples (Walder and Freedman, 1992), and has since been widely used for determination of Pb isotope ratios in airborne PM samples. Pb isotope ratios have been measured in a number of recent studies in a variety of locations to investigate the origin of airborne Pb (Knowlton and Moran, 2010; Noble et al., 2008; Hsu et al., 2006; Widory, 2006). Shotyk and Krachler (2010) also used Pb isotopes to demonstrate that the fate of Pb from runoff can be different from Pb with different origins. They observed that humus PM impacted by leaded on-road gasoline that are derived from soil surfaces are likely to be more easily transferred to sediments than Pb of other origins, with substantial amounts retained by lakes.

Recent studies have examined the use of Pb isotope ratios as a tool for source apportionment. Duzgoren-Aydin and Weiss (2008) provide caveats for using isotope ratio analyses. They point out that Pb isotope ratios may vary when Pb from several sources of different geological origins are introduced to the same location. Duzgoren-Aydin (2007) warned that the presence of a complex mixture of contaminants containing common Pb isotopes can lead to an overestimation of the contribution of one source (e.g., soil contaminated by Pb emissions from on-road gasoline) and an underestimate of another source, such as that from industry. For this reason, Cheng and Hu (2010) suggest that Pb isotope analysis only be used when the investigators are confident that the isotopic signatures of various sources differ substantially. Pb recycling and international trading may cause more blending of Pb from various sources, so that there is less heterogeneity in the Pb isotopic signatures sampled. Additionally, Cheng and Hu (2010) point out that the isotopic signature of Pb in air or soil may change over time with changing source contributions, but historical Pb isotope data are lacking. Duzgoren-Aydin and Weiss (2008) suggest the use of geographical information systems (GIS) mapping of Pb isotopic

information to help distinguish potential sources based on location of sources in addition to the sources' isotopic signature.

Gulson et al. (2007) examined the relationships between Pb isotope ratios and source apportionment metrics at urban and rural sites in New South Wales, Australia. In this study, Gulson et al. (2007) performed source apportionment with both principal component analysis (PCA) and a neural network technique called the self-organizing map (SOM) and compared results from each method with $^{206}Pb/^{204}Pb$, $^{207}Pb/^{206}Pb$, and $^{208}Pb/^{206}Pb$ obtained from PM samples, although only $^{206}Pb/^{204}Pb$ results were presented in detail. Wintertime "fingerprints" from both the PCA and SOM methods produced similarly linear relationships with $^{206}Pb/^{204}Pb$, with linearly decreasing relationships between the isotope ratios and the "secondary industry," "smoke," "soil," and "seaspray" source categories. However, the relationships of the isotope ratios with the SOM fingerprints and PCA factors, respectively, were very similar. This finding may have been due to the presence of elements such as black carbon and sulfur in several SOM fingerprints and PCA factors. The authors suggest that this might be related to the presence of several sources, which in combination result in a weak atmospheric signal. Additionally, both $PM_{2.5}$ and TSP samples were utilized for this study, and it was found that similar results were obtained for either size cut. At the urban site, they observed that the $^{206}Pb/^{204}Pb$ ratio decreased over time with increasing contributions of industrial, soil, smoke, and sea spray sources. For the most part, these sources were not substantial contributions to Pb-$PM_{2.5}$ for the rural site. As for the Tan et al. (2006) speciation study described above, no notable differences were observed between the size fractions with regard to isotopic signature.

2.4.1.6 Continuous Pb Monitoring

Development of high time resolution measurement capabilities has advantages for determining peak exposure concentrations and diurnal exposure trends. High time resolution samplers suitable for analysis after sampling by XRF and ICPMS have been developed and applied. The eight-stage Davis Rotating Unit for Monitoring (DRUM) impactor (Raabe et al., 1988; Cahill et al., 1987) collects PM samples with a cascade impactor on Mylar film substrate on a slowly rotating drum, with samples analyzed by XRF. It has been used to measure size and time resolved Pb and other elements with a time resolution of less than 6 hours using x-ray fluorescence (Cahill, 2003; Bench et al., 2002). The University of Maryland Semi-continuous Elements in Aerosol Sampler (Kidwell and Ondov, 2004, 2001) uses direct steam injection to promote condensational growth of sampled PM at a high flow rate, and accumulates resulting droplets in a slurry by impaction. It has been successfully applied to measurement of Pb and other elements

by AAS (Pancras et al., 2006; Pancras et al., 2005) with a 30-minute time resolution. This approach is also suitable for ICPMS analysis. A gas converter apparatus has also been developed to improve transfer of ions to the ICPMS, including Pb, and successfully tested with outdoor air (Nishiguchi et al., 2008). The continuous emissions monitor based method (Xact) and X-ray based filter method (XFM) samples Pb and other metals continuously and analyzes the samples using non-destructive XRF (Yanca et al., 2006). The Xact compared very well with theoretical concentrations based on wind tunnel testing, but it has a very high (0.6 $\mu g/m^3$) MDL, limiting its usefulness to areas with higher concentrations.

Much of the recent progress in ambient aerosol instrumentation has been related to the development and improvement of single particle mass spectrometry (Prather et al., 1994). Preferential loss as a function of particle size is a concern with this method, but considerable effort has been devoted to optimizing transfer from atmospheric pressure down to time of flight operating pressures with minimal particle loss (Prather et al., 1994). This technique can also be considered as an effective method for real time Pb measurement in PM, including size-resolved measurements from 0.1 to 4.0 µm (Silva and Prather, 1997). Progress has continued in the development of single particle mass spectrometry to quantify elements and organic ion fragments and a number of recent applications that included (Snyder et al., 2009; Johnson et al., 2008; Bein et al., 2007; Reinard et al., 2007; Pekney et al., 2006) or specifically targeted (Salcedo et al., 2010; Moffet et al., 2008a; Murphy et al., 2007) Pb measurements.

2.4.2 Network Design

Four national monitoring networks collect data on Pb concentrations in ambient air and report it to the Air Quality System (AQS).[1] State and local agencies carry out the monitoring at state and local monitoring stations (SLAMS) using FRMs and FEMs and report data to these national networks, which have been established for various purposes. Although these data may be used for other scientific purposes, the SLAMS network is designed primarily with the goal of evaluating compliance with the Pb NAAQS. In addition to FRM monitoring, Pb is also measured within the Chemical Speciation Network (CSN), IMPROVE, and the NATTS networks as described in Section 2.4.2.2. Measurements among these networks are not directly comparable in all cases because of method differences, including the PM size range sampled (TSP, PM_{10}, or $PM_{2.5}$).

[1] The Air Quality System (AQS) is EPA's repository of ambient air quality data. AQS stores data from over 10,000 monitors, 5,000 of which are currently active (http://www.epa.gov/ttn/airs/airsaqs/).

2.4.2.1 NAAQS Monitoring Network

Monitors in the SLAMS network include predominantly those sited in compliance with regulatory requirements for the purposes of judging attainment with the NAAQS. For this purpose, these sites employ FRM samplers coupled with FRM/FEM analysis methods. At the time of the last review, there were approximately 250 sites operating in this network, although analyses at the time indicated incomplete coverage of the larger stationary sources of Pb (U.S. EPA, 2007h). As a result of the review, the Pb NAAQS monitoring requirements were revised. These revisions, some aspects of which were finalized in 2008 and the remainder in December 2010, call for expanded monitoring at both source-oriented and non-source-oriented sites (75 FR 81126, 40 CFR part 58, Appendix D, Section 4.5 to Part 58). Source-oriented monitoring sites are required near sources of Pb air emissions which are expected to or have been shown to contribute to ambient air Pb concentrations in excess of the NAAQS. At a minimum there must be one source-oriented site located to measure the maximum Pb concentration in ambient air resulting from each non-airport Pb source estimated to emit Pb at a rate of 0.50 or more tons/year and in locations near those airports at which activities associated with the use of leaded aviation fuel are estimated to result in Pb emissions at a rate of 1.0 or more tons per year.[1] The emission monitoring threshold was established to ensure monitoring near Pb air sources with the greatest potential to cause ambient air concentrations to exceed the Pb NAAQS.[2] The Pb NAAQS measurements required at source-oriented sites must be as Pb-TSP but measurements at non-source oriented sites may be as Pb-TSP or Pb-PM_{10} (75 FR 81126).

Monitoring agencies are also required to conduct non-source-oriented Pb monitoring at each National Core multipollutant monitoring network (NCore)[3] site in a Core Based Statistical Area (CBSA) with a population of 500,000 or more. While non-source-oriented monitoring data can be used for purposes of NAAQS attainment designations, the main objective for non-source-oriented monitoring is to gather information on

[1]The requirement for monitoring near sources emitting 0.5 tons/year or more may be waived if it can be shown that the source will not contribute to a maximum 3-month average Pb concentration in ambient air in excess of 50 percent of the NAAQS level based on historical monitoring data, modeling, or other means (40 CFR, part 58, Appendix D, Section 4.5(a)(ii)).

[2] EPA Regional Administrators may require additional monitoring beyond the minimum requirements where the likelihood of Pb air quality violations is significant. Such locations may include those near additional industrial Pb sources, recently closed industrial sources, airports where piston-engine aircraft emit Pb and other sources of re-entrained Pb dust (40 CFR, part 58, Appendix D, Section 4.5(c).

[3] NCore is a new network of multipollutant monitoring stations intended to meet multiple monitoring objectives. The NCore stations are a subset of the SLAMS network are intended to support long-term trends analysis, model evaluation, health and ecosystem studies, as well as NAAQS compliance. The complete NCore network consists of approximately 60 urban and 20 rural stations, including some existing SLAMS sites that have been modified for additional measurements. Each state will contain at least one NCore station, and 46 of the states plus Washington, D.C., will have at least one urban station.

neighborhood-scale Pb concentrations that are typical in urban areas so to better understand ambient air-related Pb exposures for populations in these areas.

Spatial scales defined for Pb monitoring range from microscale to neighborhood scale, with the most important spatial scales for source-oriented sites to effectively characterize emissions from point sources being microscale and middle scale, and the most important scale for non-source-oriented sites to characterize typical Pb concentrations in urban areas being neighborhood scale [40 CFR Part 58, Appendix D, 4.5(d)]:

- Microscale: This scale is intended to typify areas in close proximity to Pb point sources where it may represent an area impacted by the emissions plume with dimensions ranging from several meters up to about 100 meters.
- Middle Scale: This scale is described as generally representing Pb air quality levels in areas up to several city blocks in size with dimensions on the order of approximately 100 meters to 0.5 km.
- Neighborhood Scale: This scale is to characterize concentrations throughout some relatively uniform land use areas with dimensions in the 0.5 to 4.0 km range. Where a neighborhood site is located away from immediate Pb sources, the site may be very useful in representing typical air quality values for a larger residential area, and therefore suitable for population exposure and trends analyses.

Source oriented monitors near sources estimated to emit 1.0 tons/year Pb were required to be operational by January 1, 2010, and the remainder of the newly required monitors, including the non-source-oriented NCore sites, were required to be operational by December 27, 2011 (75 FR 81126). Based on implementation of the December 2010 Pb network requirements as of late 2012, the Pb NAAQS monitoring network consists of approximately 260 required monitors. Figure 2-14 shows the geographic distribution of Pb NAAQS monitors in the current Pb NAAQS monitoring network. This includes monitors that previously existed and are still in operation, along with those that are newly required.

With the December, 2010 regulations, EPA also required one year of Pb-TSP (FRM) monitoring near 15 airports in order to gather additional information on the likelihood of NAAQS exceedances near airports due to the combustion of leaded aviation gasoline (75 FR 81126). These airports were selected based on three criteria: annual Pb inventory between 0.50 tons/year and 1.0 tons/year, ambient air within 150 meters of the location of maximum emissions (e.g., the end of the runway or run-up location), and airport configuration and meteorological scenario that leads to a greater frequency of operations from one runway. These characteristics were selected because they are expected, collectively, to identify airports with the highest potential to have ambient Pb concentrations approaching or exceeding the Pb NAAQS. Data from this monitoring study will be used to assess the need for additional Pb monitoring at airports. These 15

sites (Figure 2-15 and Table 2-4) were required to be operational no later than December 27, 2011. Evaluating the air quality impact of piston aircraft operations includes consideration of the seasonal variation in activity by these aircraft. At some of the most active general aviation airports in the country, spring and summer operations (by piston aircraft) can increase as much as 73% over operations in the fall and winter, while at other airports, piston aircraft activity is more consistent throughout the year.

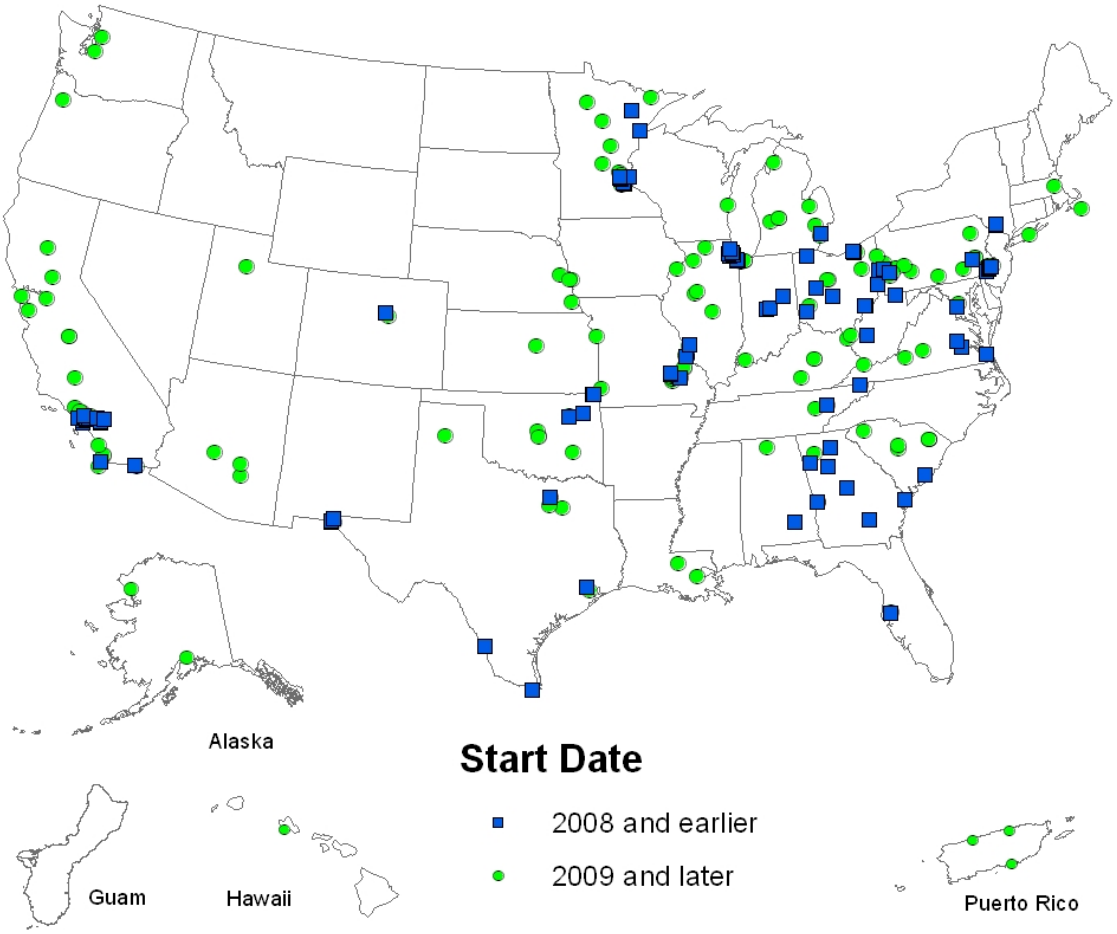
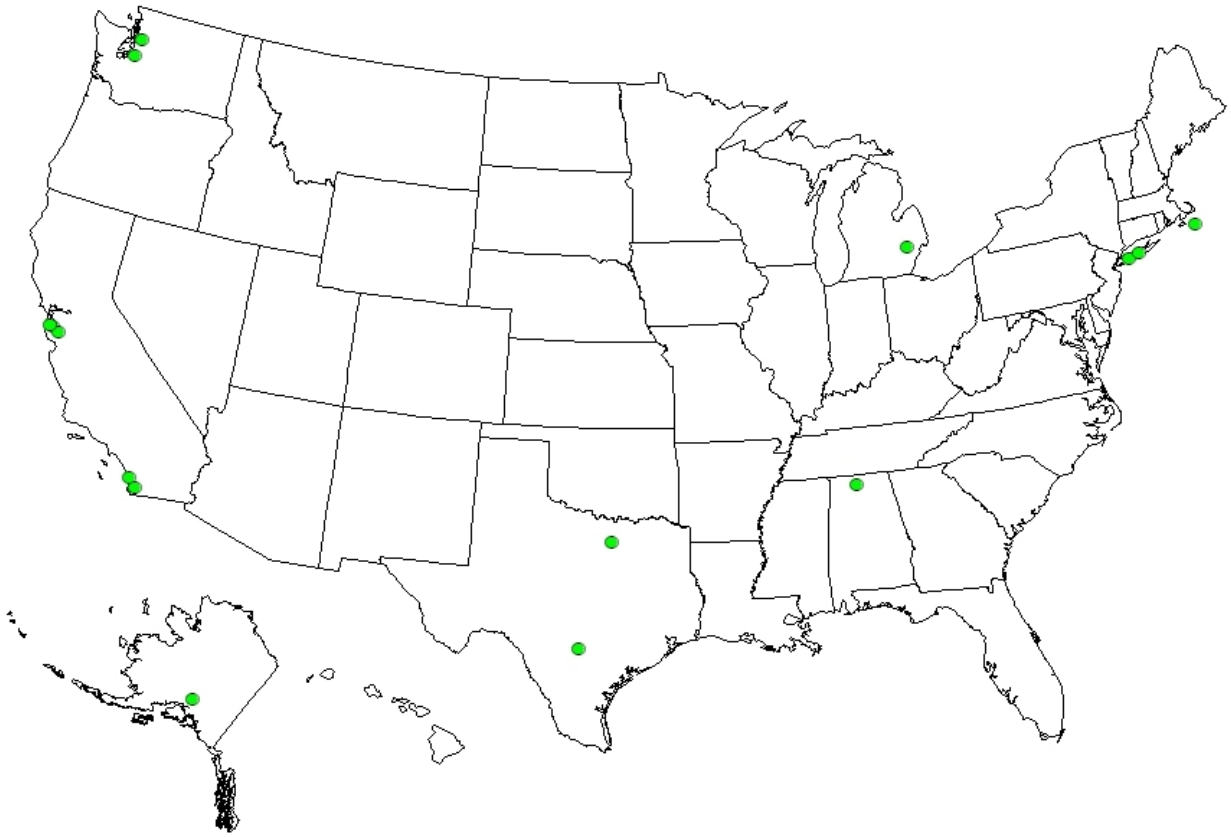


Figure 2-14 Map of monitoring sites in current Pb NAAQS monitoring network.



Quality assured results of this study were not available in time for this assessment. Note that the two Santa Clara County, CA airports are not distinguishable on the map.

Figure 2-15 Fifteen U.S. locations where a study is currently being performed on airport Pb emissions.

Table 2-4 List of 15 airports included in the airport study.

Airport	County, State
Merrill Field	Anchorage, AK
Pryor Field Regional	Limestone, AL
Palo Alto Airport of Santa Clara County	Santa Clara, CA
Reid-Hillview	Santa Clara, CA
McClellan-Palomar	San Diego, CA
Gillespie Field	San Diego, CA
San Carlos	San Mateo, CA
Nantucket Memorial	Nantucket, MA
Oakland County International	Oakland, MI
Republic	Suffolk, NY
Brookhaven	Suffolk, NY
Stinson Municipal	Bexar, TX
Northwest Regional	Denton, TX
Harvey Field	Snohomish, WA
Auburn Municipal	King, WA

2.4.2.2 Other Pb Monitoring Networks

In addition to FRM monitoring, Pb is also measured within the Chemical Speciation Network (CSN), Interagency Monitoring of Protected Visual Environments (IMPROVE), and the National Air Toxics Trends Station (NATTS) networks. Pb in $PM_{2.5}$ is monitored as part of the CSN and IMPROVE networks, and Pb in PM_{10} as a part of the National Air Toxics Trends (NATTS) networks (Figure 2-16 and Figure 2-17). These networks are designed to meet different objectives than those of the Pb NAAQS monitoring network.

The purpose of the CSN is to monitor $PM_{2.5}$ species to assist in understanding $PM_{2.5}$ chemistry and for spatial and temporal analyses including annual, seasonal, and sub-seasonal trends (http://www.epa.gov/ttn/amtic/specgen.html). The CSN consists of about 50 long-term trends sites (commonly referred to as the Speciation Trends Network or STN sites) and about 150 supplemental sites, all operated by state and local monitoring agencies. Higher spatial and temporal resolution of the CSN facilitates increased utility in the scientific community, and the data from the CSN also assist states in formulating their emission control strategies, even if the network is not compliance-oriented. Pb is one of

33 elements in $PM_{2.5}$ collected on Teflon filters every third day and analyzed by energy dispersive XRF spectrometry.

In the IMPROVE networks, $PM_{2.5}$ monitors, operated by the National Park Service, largely with funding by EPA, are placed in "Class I" areas (including National Parks and wilderness areas) and are mostly in rural locations. IMPROVE monitoring is intended to establish current visibility conditions, track changes in visibility and determine causal mechanisms of visibility impairment in 156 national parks and wilderness areas. There are 110 formally designated IMPROVE sites and approximately 80 additional sites at various urban and rural areas, informally treated as part of this network and operating under IMPROVE protocols. At these sites, Pb in $PM_{2.5}$ is determined by XRF (UC, 1995).

The NATTS network is designed to monitor concentrations of hazardous air pollutants (HAPs). The NATTS is intended to provide model input, to observe long-term trends in HAP concentrations, and to examine emission control strategies. The NATTS network measures several inorganic HAPs in PM_{10}, along with several volatile organic compounds (VOCs), carbonyls, and polycyclic aromatic hydrocarbons (PAHs). It is operated by state and local agencies and has less extensive national coverage than the other Pb monitoring networks. PM_{10} is collected either by high volume sampling with a quartz fiber filter or low volume sampling with a PTFE filter following EPA Compendium Method IO-3.5 (U.S. EPA, 1999). Pb is one of seven core inorganic HAPs collected on Teflon filters and analyzed by ICPMS. As of 2012, the network consisted of 25 monitoring stations that measure Pb-PM_{10}, including 20 urban and 5 rural stations operating on a one in six day sampling frequency (Figure 2-17).

Pb monitoring is also conducted at NCore monitoring sites. Monitoring for Pb-$PM_{2.5}$ is currently being conducted at NCore sites as part of the larger CSN (see Section 2.4.2.1). Non-source-oriented Pb monitoring required at the 51 NCore sites with a population of 500,000 or more (shown in Figure 2-17) is generally monitored in Pb-PM_{10}. Methods for Pb in $PM_{10-2.5}$ are being developed as part of the $PM_{10-2.5}$ speciation pilot project and may be implemented at some NCore sites in the future. As shown in Figure 2-17, there are some cases where states have addressed their NATTS and NCore Pb monitoring needs with the use of a single monitoring site or may have nearby sites. In addition to the NATTS network and NCore sites, as of 2012, states were collecting Pb-PM_{10} at an additional 22 sites (most as part of the Urban Air Toxics Monitoring program).

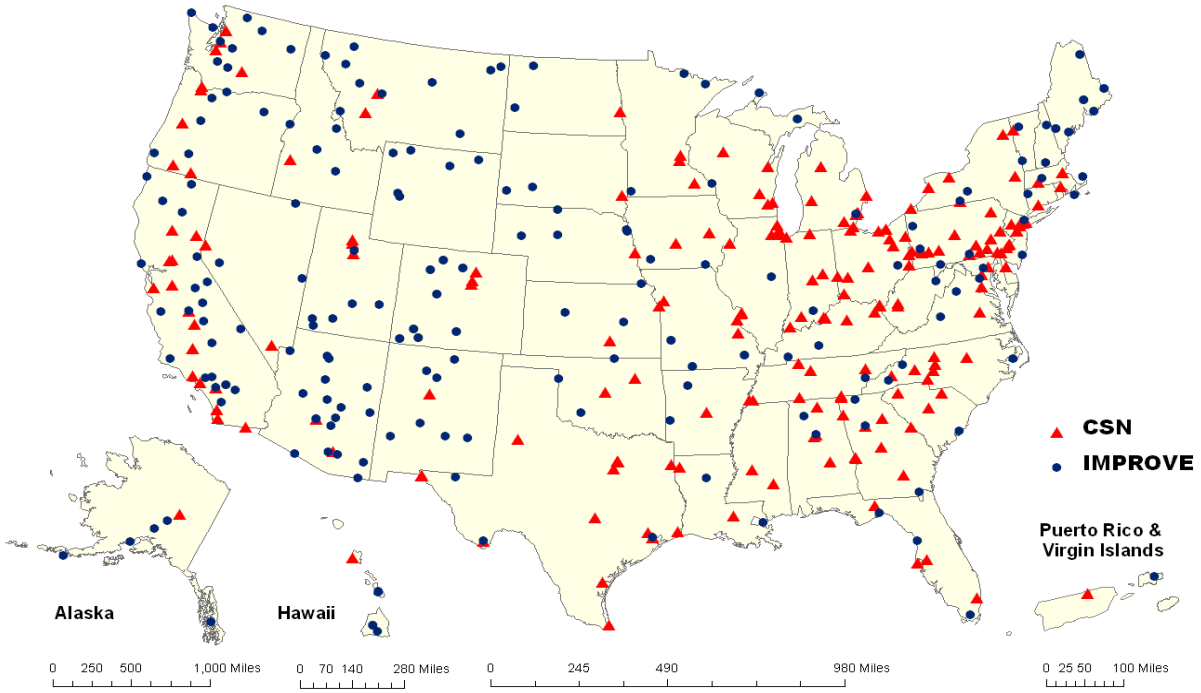
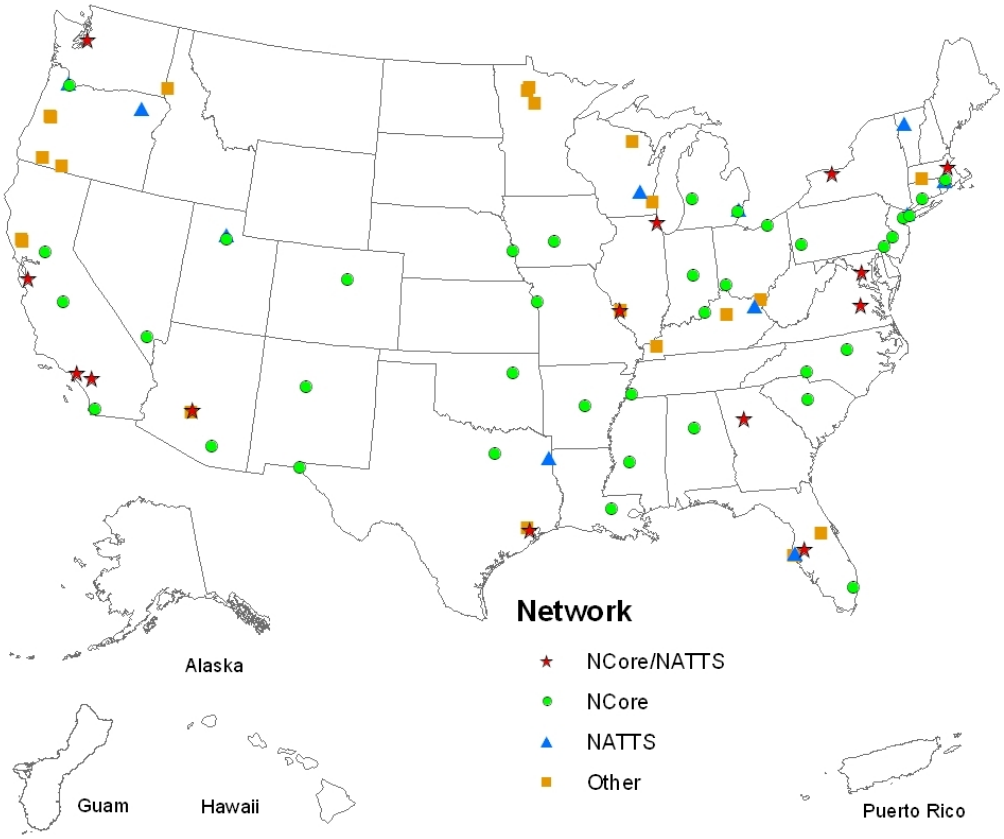


Figure 2-16 Pb-$PM_{2.5}$ monitoring sites for CSN and IMPROVE networks.



Note: Presented on this map are all NCore sites where FRM/FEM Pb monitoring is required along with other sites actively monitoring Pb-PM_{10} (by any method) based on having 2012 data in AQS as of September, 2012.

Figure 2-17 Pb-PM_{10} monitoring sites for NATTS network.

2.5 Ambient Air Pb Concentrations

The following section synthesizes ambient air Pb concentration data obtained during the years 2008-2010 with data from studies in the literature presenting Pb concentrations under varied source influences. The 3-month average ambient air Pb concentrations presented here were created using a simplified approach of the procedures detailed in 40 CFR part 50 Appendix R and, as such, cannot be directly compared to the Pb NAAQS for determination of compliance with the Pb NAAQS. For the purpose of analyses within this ISA, monitors were initially designated to be source-oriented if either (1) they were designated in AQS as source-oriented, (2) they were located within one mile of a 0.5 ton/year or greater source as noted in the 2005 NEI (U.S. EPA, 2008a), or (3) they were located within one mile of a 0.5 tons/year or greater source as noted in the 2008 NEI

(U.S. EPA, 2011a). The remainder of Pb-TSP FRM monitors reporting to the AQS were classified as non-source-oriented.[1] For this analysis, 120 Pb-TSP FRM monitors were considered source-oriented, while 184 were considered to be non-source-oriented. However, the number of source-oriented and non-source-oriented monitors differed for each analysis year because there were changes in monitor siting. Summary information is presented within this section, and detailed data are included the Chapter 2 Appendix (Section 2.8, Supplemental Material) to this chapter.

2.5.1 Spatial Distribution of Air Pb

2.5.1.1 Variability across the U.S.

This section presents nationwide Pb concentration data measured using source-oriented and non-source-oriented Pb-TSP FRM monitors from 2008-2010 and PM_{10} and $PM_{2.5}$ monitors for 2007-2009. The source and non-source-oriented Pb-TSP FRM monitors present data pertaining to compliance with the current level of the NAAQS. Pb-PM_{10} data obtained from the NATTS network and Pb-$PM_{2.5}$ data from the CSN are presented in the Chapter 2 Appendix (Section 2.8) to illustrate the nationwide distribution of Pb concentration in different classes of particle size. This information is useful to develop a sense of variability in Pb concentrations at a national scale.

Concentrations of Pb Measured using Pb-TSP Monitors (Source-Oriented and Non-Source-Oriented Monitors)

Maximum 3-month average Pb concentrations[2] were calculated for source-oriented Pb-TSP monitors for 50 counties across the U.S. (1.6% of U.S. counties) during the period 2008-2010. Figure 2-18 illustrates that the level of the NAAQS was exceeded in twenty counties where source-oriented monitoring was performed. The mean exceeded the level of the NAAQS and was skewed toward the 75th percentile of the distribution, indicating that highest ambient air Pb concentrations are near a small subset of the monitors. Upper 90th percentile ambient air Pb concentrations for 2008-2010 occurred in

[1] Following this initial classification, staff from the EPA Regional offices tasked with acting as liaisons to the states reviewed all monitors listed to fall within their Regions and reported any discrepancies between the initial classification and ground observations of the sites made by EPA Regional or state staff. The source and non-source monitor listing was edited accordingly. The definition of source-oriented monitoring is applied flexibly with input from regions in this ISA because 2008 data were obtained before the latest monitor designation requirements were implemented.

[2] Maximum 3-month average Pb concentrations are calculated as the maximum 3-month average of 3 consecutive monthly averages within the 2008-2010 time period.

Pike Co., AL, Los Angeles Co., CA, Iron Co., MO, Jefferson Co., MO, and Collin Co., TX. Summary statistics for the monitor-specific one-month and three-month averages are presented in Table 2-5, and detailed statistics for the one-month and three-month averages are provided in Table 2-12, Table 2-14, Table 2-16, and Table 2-18 in the Chapter 2 Appendix (Section 2.8).

Maximum 3-month average Pb concentrations were calculated for non-source-oriented Pb-TSP monitors for 47 counties across the U.S. (1.5% of U.S. counties) during the period 2008-2010. Figure 2-19 illustrates that the level of the NAAQS was never exceeded at non-source-oriented monitors. Summary statistics are presented below in Table 2-6, and detailed statistics for the one-month and three-month average and maxima non-source-oriented Pb-TSP concentrations are provided in Table 2-13, Table 2-15, Table 2-17, and Table 2-19 in the Chapter 2 Appendix (Section 2.8).

Table 2-5 Summary data for source-oriented Pb monitors across the U.S., 2008-2010.

	Mean, µg/m^3	Median, µg/m^3	95th%, µg/m^3	99th%, µg/m^3	Max, µg/m^3
Monthly	0.20	0.063	0.86	1.6	4.4
3-mo rolling avg	0.21	0.079	0.88	1.6	2.9

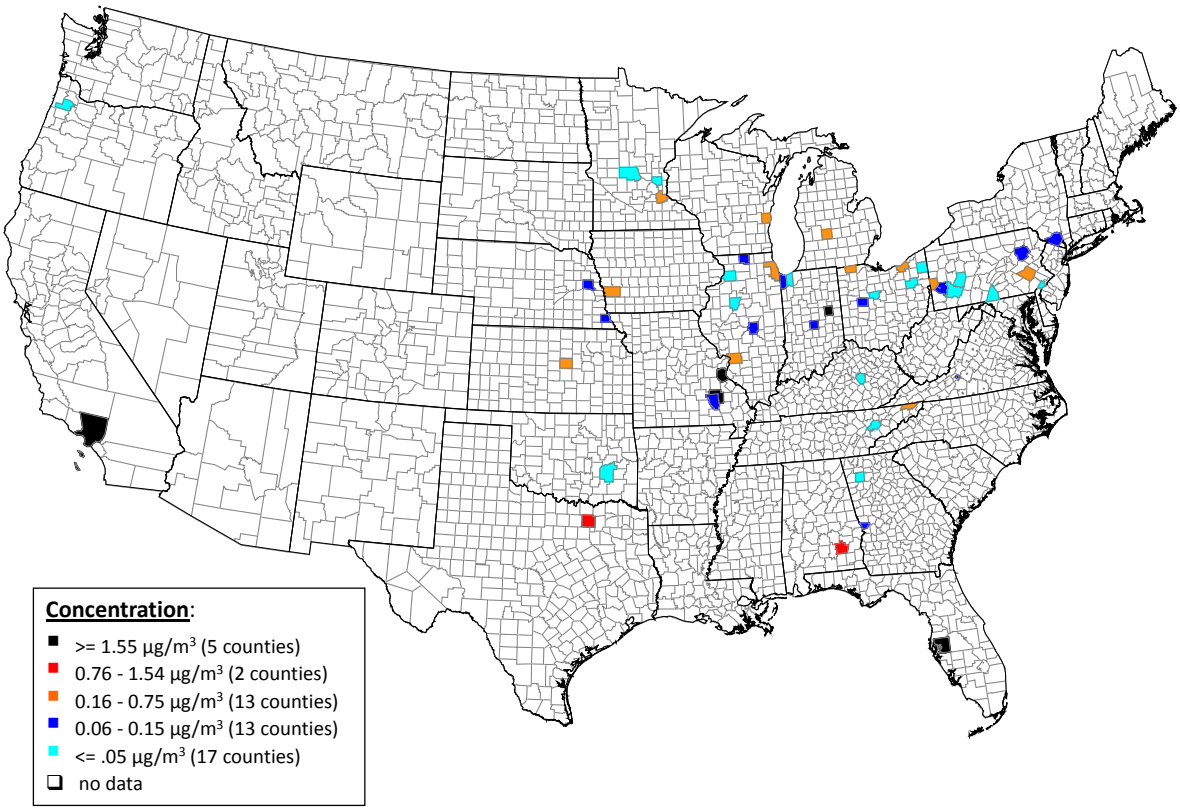


Figure 2-18 Highest county-level source-oriented Pb-TSP concentrations (µg/m^3), maximum 3-month average, 2008-2010.

Table 2-6 Summary data for non-source-oriented Pb monitors across the U.S., 2008-2010.

	Mean, µg/m^3	Median, µg/m^3	95th%, µg/m^3	99th%, µg/m^3	Max, µg/m^3
Monthly	0.012	0.010	0.040	0.052	0.14
3-mo rolling avg	0.012	0.010	0.037	0.048	0.073

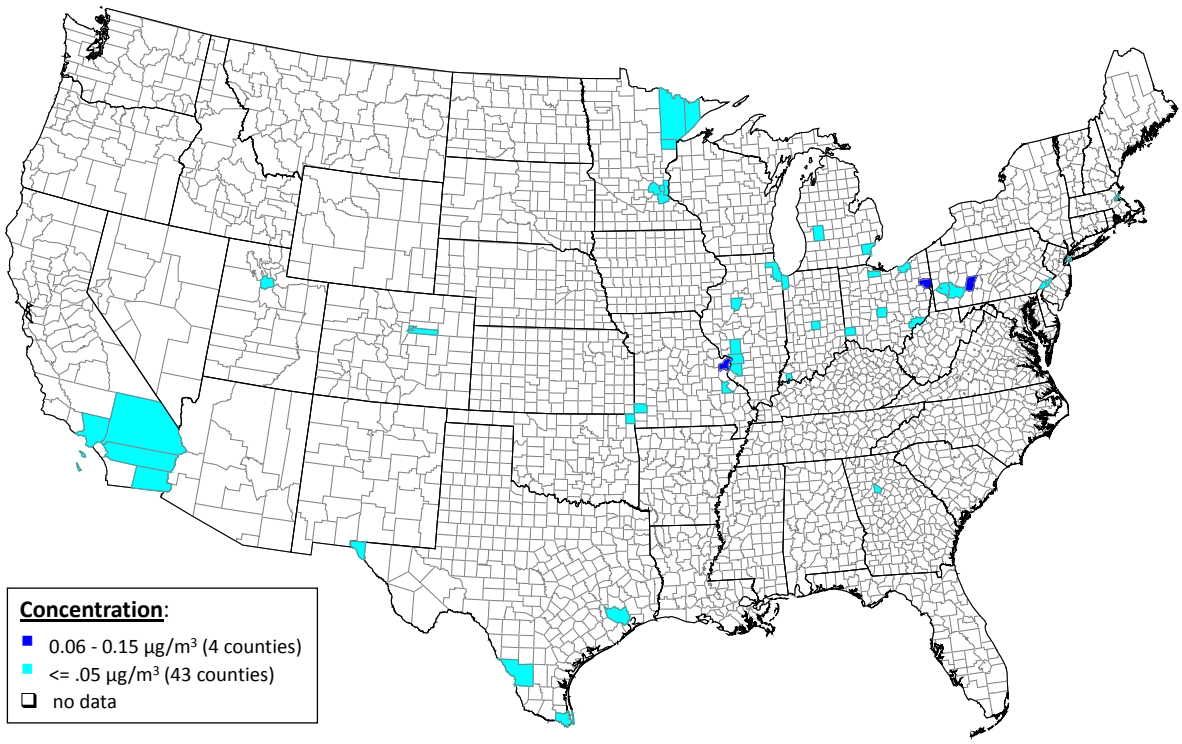


Figure 2-19 Highest county-level non-source-oriented Pb-TSP concentrations (µg/m^3), maximum 3-month average, 2008-2010.

2.5.1.2 Intra-urban Variability

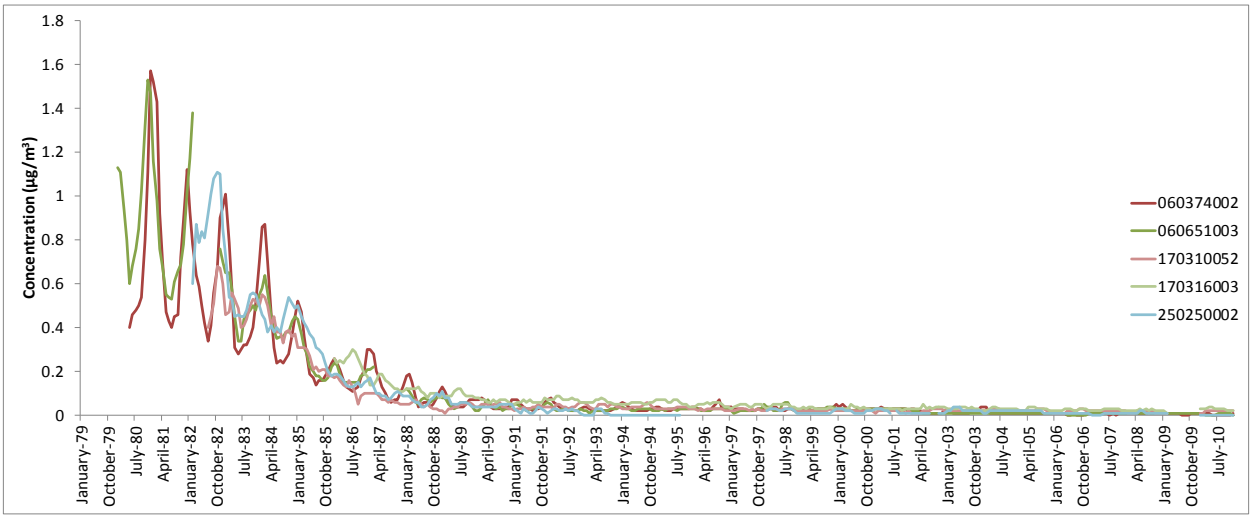
Intra-urban variability is defined as the variation in Pb concentration across an urban area. Because the source characteristics and size distribution of particle-bound Pb can vary considerably in urban areas, spatial variability of Pb concentrations in urban areas may also be high. Moreover, larger Pb-PM tends to settle quickly over short distances after becoming airborne; short settling distances also contribute to high spatio-temporal variability in ambient air Pb concentrations. Such variability may not be detected if one or a small number of central site monitors is in use, so cities with multiple monitors are used to characterize intra-urban variability.

Data for intra-urban variability in six U.S. counties are presented in Section 2.8.2 of the Chapter 2 Appendix. When collectively reviewing the data from the six counties, it became apparent that spatial and temporal variability of Pb-PM concentrations were commonly high compared for example with $PM_{2.5}$, which tends to have fairly homogenous concentrations over urban areas because it is subject to secondary formation. Variability was high for areas that included a Pb source, with high concentrations downwind of the sources and low concentrations at areas far from sources. When no large sources of Pb were present, variability of Pb concentrations were lower, and more data were observed to lie below the MDL. For example, Los Angeles County, CA data illustrated very high concentrations adjacent to a Pb recycling facility, but non-source-oriented concentrations were well below the level of the NAAQS at all times, including at sites near roads. As described in Section 2.3, PM size distribution influences how far the particle will travel upon initial emission or resuspension before being deposited. Meteorology, nature of the sources, distance from sources, and positioning of sources with respect to the monitors all appeared to influence the level of concentration variability across time and space.

Airborne Pb near Roads

Five monitors, described in Table 2-7, were selected from the TSP network to examine Pb concentrations in the near road environment. These monitors were selected because they are located in the vicinity of major roadways in urban areas with different characteristics and because they each have long-term data available. Further, based on reviews of emissions inventory information as well as satellite image searches, these sites are not known to be near metals-related industrial sites. Time series of Pb-TSP monthly concentration for all five monitors are shown in Figure 2-20. The annual average over the two sites that were reporting data in 1980 was 0.90 $\mu g/m^3$. This Pb-TSP concentration from 1980 likely reflected the influence of Pb emissions from leaded automobile gasoline (see Figure 2-7 for annual national consumption of leaded motor vehicle gasoline). By

1986, when all five monitors were reporting data, the annual average of Pb-TSP concentration over all five monitors dropped to 0.18 µg/m^3. Over 2001-2010, the annual average Pb-TSP concentration over all sites was 0.02 µg/m^3 with a standard deviation of 0.01 µg/m^3. The highest 2008-2010 design value was 0.04 µg/m^3, which occurred at the Chicago site (17-031-6003) located less than 10 meters to Interstate I-290 at a monitor height of 2 meters AGL. The multi-site average was not substantially larger than the maximum three-month rolling average of 0.012 µg/m^3 for non-source-oriented monitors for the period 2008-2010, and the Pb-TSP concentration varied little over the period 2001-2010. Note that the monitor heights were 2-6 meters AGL, which may be higher than the vertical distance likely traveled by some particles (depending on particle size) following initial resuspension (see Section 2.3.1.3).



Note: Monitor IDs from Table 2-7: Los Angeles, CA: 06-037-4002 (dark red); Riverside, CA: 06-065-1003 (dark green); Two from Cook, IL: [17-031-0052 (light red), and 17-031-6003 (light green)]; and Suffolk, MA: 25-025-0002 (blue).

Figure 2-20 Time series of monthly average Pb-TSP concentration at five near-road monitors.

Table 2-7 Sample of U.S. near-road Pb-TSP monitors.

County, State	Site ID	Latitude	Longitude	2008-2010 Design Value ($\mu g/m^3$)[a,b]	Monitor Height (m AGL)	Distance from Roads	Surrounding Area
Los Angeles, CA	06-037-4002	33.82376	-118.18921	0.01	6	500 m to Interstate I-405 (San Diego Freeway), 10 m to Long Beach Blvd	High intensity residential, urban
Riverside, CA	06-065-1003	33.94603	-117.40063	0.01	4	Within 20 m of intersection of Magnolia and Arlington Ave.	High intensity residential, mixed use urban
Cook, IL	17-031-0052	41.96548	-87.749928	0.02[c]	5	Near to intersection of Interstates I-90 and I-94, 80 m to Interstate I-90, 200 m to Interstate I-94, 70 m to railroad	Located at public utilities water pumping station, high density residential urban
Cook, IL	17-031-6003	41.872202	-87.826165	0.04[c]	2	Less than 10 m to Interstate I-290 (Dwight D. Eisenhower Expressway)	Parking lot of Circuit Court of Cook County, 75% surrounded by Concordia Cemetary
Suffolk, MA	25-025-0002	42.348873	-71.097163	0.02[c]	5	95 m to Interstate I-90, inside median of Commonwealth Ave.	High intensity urban, mixed use residential and commercial

[a]The level of the 2008 NAAQS for Pb is 0.15 $\mu g/m^3$ not to be exceeded in any 3-month period. The design value for the 2008 Pb NAAQS is the maximum rolling 3-month Pb-TSP average within the 3-year design period.

[b]The design values shown here are computed for the latest design value period using Federal Reference Method (FRM) or equivalent data reported by States, Tribes, and local agencies to EPA's AQS as of 7/12/2011. Concentrations flagged by States, Tribes, and local agencies as exceptional events (e.g., high winds, wildfires, volcanic eruptions, construction) and concurred by the associated EPA Regional Office are not included in the calculation of these design values. Although the indicator for the 2008 Pb NAAQS is Pb-TSP at "local conditions" (i.e., actual temperature and pressure; parameter 14129), 2008 Pb-TSP data reported in "standard temperature and pressure" (i.e., 25 ° C, 760 mmHg; parameter 12128) are also considered valid for NAAQS comparisons and related attainment/nonattainment determinations if the sampling and analysis methods that were utilized to collect that data were consistent with previous or newly designated FRMs or FEMs and quality assurance requirements were met.

[c]Fewer than 36 rolling 3-month Pb-TSP average data are available at this site for this 3-year period; the value shown here is the highest valid 3-month mean.

Airborne Pb near Airports

There have been only a few studies of air Pb concentrations near airports, but they have generally demonstrated consistent results. Levin et al. (2008) summarized findings from measurements at Buttonville Airport near Toronto, Canada that median air Pb-PM_{10}

levels were not substantially higher than average reported background levels (0.01 μg/m^3 versus 0.007 μg/m^3) (Conor Pacific Environmental Technologies Inc, 2000), although the Buttonville analysis averaged upwind and downwind data. The maximum 24-hour concentration measured in this 10-day study was 0.13 μg/m^3. The Illinois report noted that air Pb concentrations were elevated downwind of O'Hare airport compared with upwind levels (Illinois Environmental Protection Agency, 2002). Carr et al. (2011) also noted elevated Pb concentrations immediately downwind of the runway when studying Pb concentrations at the Santa Monica Airport and surrounding neighborhood in Santa Monica, CA in 2009. Twenty-four hr average concentrations at the downwind location were higher than concentrations measured immediately upwind of the runway (winter: 0.040 μg/m^3 versus 0.0075 μg/m^3; summer: 0.049 μg/m^3 versus 0.0040 μg/m^3). Summer measurements also included a residential neighborhood 100 meters further downwind, which were still higher than upwind (0.033 μg/m^3 versus 0.004 μg/m^3). Modeling results from Carr et al. (2011) also suggest that three-month average Pb concentrations above local background extended beyond the airport property and that the preflight runup check, taxi, and takeoff emissions were the most important contributors to Pb concentrations. This airport had a Pb emissions inventory of 0.3 tons/year (U.S. EPA, 2011a), which is below the threshold for airports for requiring consideration of Pb NAAQS compliance monitoring; the monitoring regulations also specify the placement of monitors in locations where available information indicates the potential for exceedance of the NAAQS (see Section 2.4.2.1).

Airborne Pb at Urban and Rural Sites

A number of studies characterizing airborne Pb-bearing PM distribution at the neighborhood scale suggest that spatial variability in Pb concentrations is related to local sources. Yu et al. (2011) measured Pb-PM_{10} concentration using a four-channel PM sampler (Thermo Scientific) at four roof-top sites (10-13 meters AGL) within Paterson, NJ: (1) background, (2) near-road (within 0.8 km of two major roads), (3) industrial (within 1 km of three metal processing facilities), and (4) commercial. Coefficient of variation (CV), defined as the standard deviation of site measurements divided by the average) was 31.3%, with concentrations ranging of 5.61 ng/m^3 (near road), 6.48 ng/m^3 (industrial), and 6.58 ng/m^3 (commercial), compared with 2.95 ng/m^3 at the background site. Harrison and Yin (2010) also noted that median urban background Pb concentrations were elevated compared with rural background (urban: 13.9 ng/m^3; rural: 8.0 ng/m^3). Martuzevicius et al. (2004) examined the spatial variability of Pb-$PM_{2.5}$ samples obtained in the greater Cincinnati, OH area at 6 urban, 4 suburban, and 1 rural site using Harvard $PM_{2.5}$ Impactors. They found that Pb-$PM_{2.5}$ had a CV of 33.8%, compared with a CV for $PM_{2.5}$ of 11.3% over all sites. Average Pb-$PM_{2.5}$ concentration among the sites varied

from 1.79-28.4 ng/m^3. Martuzevicius et al. (2004) suggested that differences between mass and Pb spatial variability implied that Pb originated primarily from local sources. Sabin et al. (2006a) measured Pb-PM with a Noll Rotary Impactor having an upper cutpoint of 29 μm and found that urban concentrations ranged from 2.2 to 7.4 ng/m^3 with a CV of 40%. In contrast, a rural location had a concentration of 0.62 ng/m^3. Li et al. (2009a) collected $PM_{2.5}$ with a Harvard Impactor and observed that Pb concentration in $PM_{2.5}$ samples was 2.2-3.0 times higher near a bus depot than next to a rural-suburban road; in this study, the authors provided ratios but not actual concentrations. Ondov et al. (2006) measured Pb-$PM_{2.5}$ concentration at three Baltimore sites using an FRM. Average Pb-$PM_{2.5}$ concentrations at the different sites were 8.3 ng/m^3, 7.2 ng/m^3, and 1.9 ng/m^3, with the two higher concentration sites located within two miles of industrial facilities. The industrial sites include a major steel plant; several chemical manufacturing plants; and incinerators for municipal waste, medical waste, and sludge. Although these concentrations are low, they agree with the body of literature to suggest that intra-urban variability is most strongly related to source type, strength, and location.

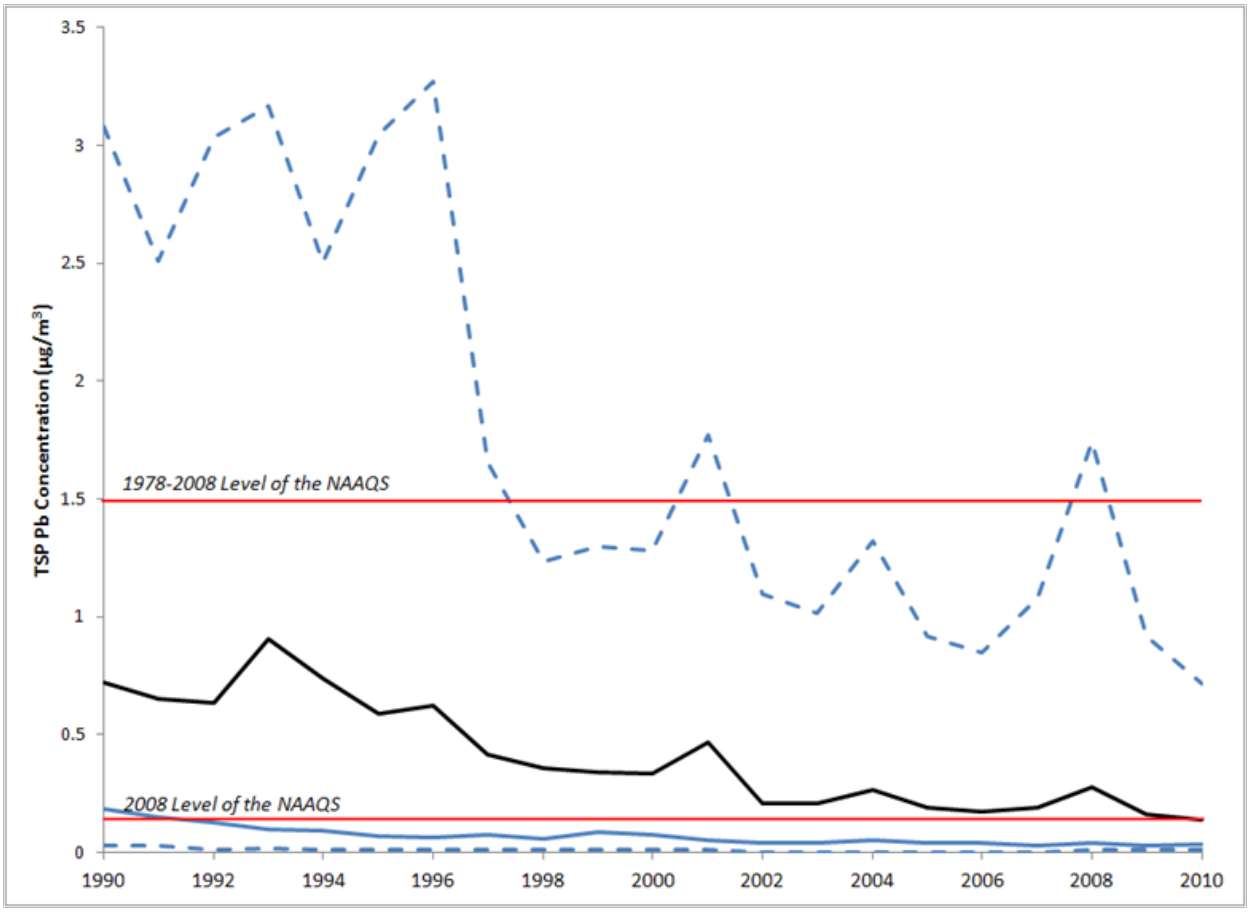
2.5.2 Temporal Variability

The following sections present data for multi-year trends and seasonal variability of Pb concentrations on a nationwide basis. The data presented here provide information on the success of Pb reduction efforts over past decades as well as on areas for continued attention with respect to Pb monitoring. The multi-year trends illustrate changes in air Pb concentrations resulting from the phase-out of leaded gasoline for automobiles and smaller reductions of industrial Pb usage. The seasonal variability plots demonstrate changes in concentration within a given year, potentially related to climate or source variation.

2.5.2.1 Multi-year Trends

Pb-TSP concentrations have declined substantially during the years 1980-2010. For source and non-source monitors combined in the trends network, the annual average of the maximum 3-month averages across 74 Pb-TSP monitors reporting air Pb concentrations over the period from 1980-2010 has dropped by 89% from 1.3 μg/m^3 in 1980 to 0.14 μg/m^3 in 2010 (n = 31); see Figure 2-21. The median maximum 3-month average concentration has declined by 97% from 0.87 μg/m^3 in 1980 to 0.03 μg/m^3 in 2010. The decline can be attributed to the phase-out of Pb antiknock agents in on-road fuel and reductions in industrial use and processing of Pb, as described in Section 2.2.1.

Average concentrations in these calculations are heavily influenced by the source-oriented monitors in the network.



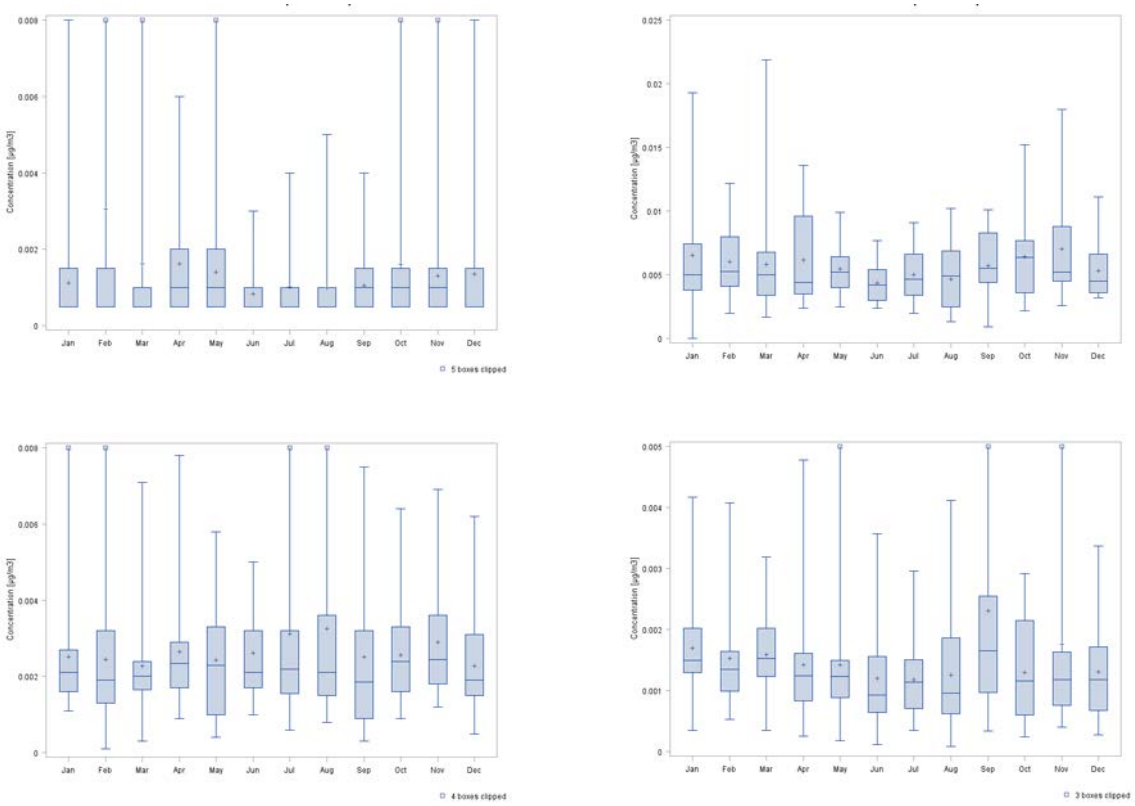
Note: Annual average of maximum 3-month average Pb-TSP concentrations is shown by the solid black line, annual median of maximum 3-month average concentrations is shown by the solid blue line, and the 10th and 90th percentiles are shown by the dashed lines.

Figure 2-21 National trends in Pb concentration (µg/m^3), 74 trends sites, 1990-2010.

2.5.2.2 Seasonal Variations

This section outlines seasonal variability among Pb monitors. Seasonal variation may provide insight related to differential influences of sources and climate throughout a year. Figure 2-22 illustrates average monthly trends in Pb-$PM_{2.5}$ at four IMPROVE sites: Lake County, CA (060333010), Bronx County, NY (360050110), Monroe County, NY (360551007), Chittenden, VT (500070007). In each plot, some month-to-month

variability is evident. Seasonal trends, with peaks in median,75th, and 95th percentiles in the spring and fall, are apparent in the Bronx and Chittenden sites. Variability in the median values is less pronounced. These sites do not illustrate national trends, but they do collectively suggest that there can be seasonal variation in ambient air Pb concentration within sites consistent with findings from the 1970s and 1980s (U.S. EPA, 1986a). National trends in monthly concentrations, provided in the Chapter 2 Appendix (in Figure 2-58, Figure 2-59, Figure 2-60, and Figure 2-61), do not illustrate variability because inter-site variability is averaged out.



Legend: Top left panel: Lake County, CA 060333010, Top right panel: Bronx County, NY 360050110, Bottom left panel: Monroe County, NY 360551007, and Bottom right panel: Chittenden, VT 500070007.

Notes: Data were not available for all years at all sites. IMPROVE sites were chosen where at least three years of data were available during 2001-2011. Boxplots are clipped at all but the Bronx site to improve illustration of the variability among the monthly interquartile ranges.

Figure 2-22 Boxplots of average monthly Pb-$PM_{2.5}$ concentrations measured at four IMPROVE sites, 2001-2010.

Other data regarding seasonal variability of ambient air Pb concentrations have been limited. Laidlaw et al. (2012) also explored the seasonal variability of Pb-$PM_{2.5}$ at four cities (Birmingham, AL, Chicago, IL, Detroit, MI, and Pittsburgh, PA) using data from the Interagency Monitoring of Protected Visual Environments (IMPROVE) network. They observed a strong seasonal pattern with elevated Pb-$PM_{2.5}$ levels in the summer compared with the winter for all four cities. Likewise, Harrison and Yin (2010) observed that winter background concentrations of Pb were 88% and 81% of summer background concentrations for urban and rural settings, respectively. In addition to the data presented above, monthly average Pb concentrations averaged across sites from the TSP, NATTS, and CSN networks are provided in the Chapter 2 Appendix (Section 2.8.3).

2.5.3 Size Distribution of Pb-Bearing PM

The diverse nature of the main source types of ambient air Pb contributes to variations in Pb-PM size distribution. Such variation in the size distribution, along with size-dependent biases in Pb-TSP collection efficiency (Section 2.4.1.1), can lead to uncertainties in the interpretation of results from Pb-PM measurements. Accordingly, depending on the locations and magnitudes of nearby sources, ambient air Pb may be 1) mainly Pb in PM_{10} and $PM_{2.5}$, for which good sampler performance is well established, 2) Pb-PM with a size distribution that ranges up to slightly larger than 10 µm, in which case the existing Pb-TSP FRM could potentially be subject to wind related bias, or 3) a Pb-PM size range that extends well above 10 µm, or too large to be efficiently collected even by an improved Pb-TSP method. In the latter case, air sampling is likely to be less representative of actual concentrations of Pb. The role of ambient air Pb size distribution on human exposure, along with the role of the size distribution of Pb in soil and dust, is described in Section 3.1.1.1.

Because atmospheric lifetime is dependent on particle size, as described in Section 2.3.1.3 and in the U.S. EPA 2009 PM ISA (2009a), TSP sampling is likely to be representative only on a very small spatial scale. Ultra-coarse particles have a sharp concentration gradient with distance from source, because coarser particles have greater settling velocities. Hence, concentrations of particles larger than 10 µm are likely to be very spatially and temporally heterogeneous compared with finer particles (U.S. EPA, 2009a; Hinds, 1999). As a consequence, in locations near sources of ultra-course particles, measurements may reflect true concentrations only in small areas in close proximity to the monitor. This issue has been thoroughly discussed in the 2006 Pb AQCD (U.S. EPA, 2006b), as well as in the 1977 Pb AQCD (U.S. EPA, 1977).

Size-selective monitoring data from AQS and the literature are examined in this section. Size distribution data enhance understanding of the relationship between sources and characteristics of airborne Pb-bearing PM and hence inform monitoring strategies. Several studies in the literature since the last review have been designed to characterize the size distribution of Pb concentrations in the vicinity of sources. In the following subsections, the currently available information is presented for locations in the vicinity of industrial sources (active and closed), near roadways, and in other urban and rural environments.

2.5.3.1 Co-located Monitoring Data Analysis

This section employs AQS data for Pb concentrations from co-located TSP, PM_{10}, and/or $PM_{2.5}$ monitors to analyze correlations and ratios of concentrations obtained from the different monitors. These data were used because relationships among the monitors provide information about the nature of Pb-bearing PM at different locations (e.g., whether the mode is in the fine or coarse fraction). Correlations indicate the extent to which the size fractions vary together in time, and ratios signify the average proportion of the smaller fraction to the larger fraction (e.g., the ratio of $PM_{2.5}$ to PM_{10} concentrations).

Estimation of the size distribution of Pb-bearing PM is possible at a limited number of monitoring sites where monitors having different size-selective cut-points are co-located. Data for correlations between concentrations at co-located monitors having different size cuts and average ratios of concentrations from these co-located monitors are available per co-location site in Table 2-26 in the Chapter 2 Appendix (Section 2.8), and a summary of these data is provided in Table 2-8. To ensure quality of the comparisons, they were limited to data from sites which had at least 30 pairs of co-located samples, with both samples above the MDL and where both monitors reported data at STP.

The collective size cut comparison data illustrate that the correlations and concentration ratios of Pb-TSP with the Pb-PM_{10} and Pb-$PM_{2.5}$ fractions are moderate, with less correspondence of Pb-$PM_{2.5}$ with Pb-TSP compared with Pb-PM_{10}. The findings indicate that, on average, 81% of Pb-TSP is in the Pb-PM_{10} fraction, 50% is in the Pb-$PM_{2.5}$ fraction, and 74% of the Pb-PM_{10} is in the Pb-$PM_{2.5}$ fraction (assuming no bias in the Pb-PM measurements, which may not be a reasonable assumption based on Section 2.4.1). However, for co-located pairs of Pb-TSP with Pb-PM_{10} or Pb-$PM_{2.5}$, the ranges of correlations and ratios were large, indicating substantial spatiotemporal variability. There appeared to be little difference between urban and suburban correlations and concentration ratios. For three co-located Pb-PM_{10} :Pb-TSP pairs in Wichita, KS, the average concentration ratios greater than one were observed. This suggests that some

portion of the particles captured by the PM_{10} sampler were not collected by the TSP sampler, perhaps as a result of instrument biases, as discussed in Section 2.4.1. Likewise, if such a bias is consistent across monitors, it is possible that, even for average ratios less than one, the average ratios would be lower if the particles were sampled more efficiently with the TSP monitor.

Table 2-8 Summary of comparison data for co-located ambient air Pb monitors.

Monitors[a]		Correlation			Average Ratio		
	N	Average	Standard Deviation	Range	Average	Standard Deviation	Range
All Sites							
Pb-PM_{10} vs. Pb-TSP	36	0.74	0.23	0.13-0.99	0.81	0.19	0.38-1.28
Pb-$PM_{2.5}$ vs. Pb-TSP	20	0.62	0.28	0.11-0.96	0.50	0.12	0.30-0.73
Pb-$PM_{2.5}$ vs. Pb-PM_{10}	28	0.91	0.12	0.50-0.99	0.74	0.06	0.59-0.90
Urban and City Center							
Pb-PM_{10} vs. Pb-TSP	18	0.76	0.20	0.40-0.99	0.80	0.12	0.57-0.99
Pb-$PM_{2.5}$ vs. Pb-TSP	12	0.58	0.31	0.11-0.96	0.50	0.09	0.36-0.62
Pb-$PM_{2.5}$ vs. Pb-PM_{10}	14	0.89	0.15	0.50-0.99	0.74	0.06	0.69-0.90
Suburban							
Pb-PM_{10} vs. Pb-TSP	18	0.73	0.26	0.13-0.96	0.81	0.25	0.38-1.28
Pb-$PM_{2.5}$ vs. Pb-TSP	8	0.69	0.22	0.31-0.91	0.51	0.15	0.30-0.73
Pb-$PM_{2.5}$ vs. Pb-PM_{10}	12	0.92	0.08	0.74-0.99	0.73	0.07	0.59-0.82

[a]Note: For comparability, they were limited to data from sites which had at least 30 pairs of co-located samples, with both samples above the MDL and where both monitors reported data at STP. N: sample size, PM: particulate matter, TSP: total suspended particulate matter.

2.5.3.2 Studies of Pb-bearing PM Size Distribution in the Literature

The size distribution of Pb-bearing PM has changed over time and by site. Table 2-9 is reproduced from Cho et al. (2011), which reviewed studies of the size distribution of Pb-bearing PM. Studies included in Cho et al. (2011) from the late 1960s to the early 1980s reported substantially higher Pb concentrations compared with current levels. Traffic-related emissions produced higher contributions from Pb-$PM_{2.5}$ compared with industrial emissions. More recent studies from the 1990s and 2000s illustrated variability in the size distribution regardless of whether the source was traffic or industrial. Cho et al. (2011) concluded that the size distribution appears to have shifted after the 1980s,

with the mode appearing to fall somewhere between 2.5 μm and 10 μm, compared with previous estimations of a primary mode smaller than 2.5 μm; however, Cho et al. (2011) maintained that additional data are needed to improve characterization of the Pb-PM size distribution. Metadata and size distribution data from cited studies are provided in Table 2-27 and Table 2-28, respectively, of the Chapter 2 Appendix (Section 2.8).

Table 2-9 Summary of studies reporting Pb size distribution in the peer-reviewed literature.

Study	Location or Site Type	Pb-TSP ($\mu g/m^3$)	Pb-PM_{10} ($\mu g/m^3$)	Pb-$PM_{2.5}$ ($\mu g/m^3$)	Pb-PM_1 ($\mu g/m^3$)	Pb-PM_{10} / Pb-TSP	Pb-$PM_{2.5}$ / Pb-TSP	Pb-PM_1 / Pb-TSP	Pb-$PM_{2.5}$ / Pb-PM_{10}
Lee et al. (1968)	Urban	–	–	–	–	–	–	0.75	–
	Suburban	–	–	–	–	–	–	0.65	–
Lee et al. (1972)	Chicago, IL	–	–	–	–	0.93	0.83	0.59	0.89
	Cincinnati, OH	–	–	–	–	1.00	0.88	0.72	0.88
	Denver, CO	–	–	–	–	0.99	0.88	0.70	0.89
	Philadelphia, PA	–	–	–	–	0.98	0.87	0.70	0.89
	St. Louis, MO	–	–	–	–	0.98	0.81	0.62	0.83
	Washington, DC	–	–	–	–	0.99	0.90	0.74	0.91
Dorn et al. (1976)[a,b]	Near a Pb smelter								
	Year	1.04	0.91	0.47	0.27	0.88	0.45	0.26	0.51
	Winter	1.76	1.69	0.84	0.46	0.96	0.48	0.26	0.50
	Summer	0.78	0.54	0.32	0.18	0.69	0.41	0.23	0.59
	Control site								
	Year	0.11	0.09	0.06	0.04	0.83	0.52	0.32	0.63
	Winter	0.10	0.10	0.07	0.04	0.93	0.70	0.36	0.75
	Summer	0.08	0.08	0.04	0.03	0.94	0.51	0.34	0.54
Alpert and Hopke (1981)	Urban	0.913	–	0.720	–	–	0.79	–	–
Holsen et al. (1993)	Urban	–	0.0257	0.0189	–	–	–	–	0.69
	Rural	–	0.0052	0.0043	–	–	–	–	0.92
	Lake Michigan	–	0.0112	0.0091	–	–	–	–	0.81
Sweet and Gatz (1998)[h]	Lake Erie	–	0.0009	0.0070	–	–	–	–	–
	Lake Michigan	–	0.0014	0.0021	–	–	–	–	–
	Lake Superior	–	0.0013	0.0031	–	–	–	–	–

Table 2-9 (Continued): Summary of studies reporting Pb size distribution in the peer-reviewed literature.

Study	Location or Site Type	Pb-TSP ($\mu g/m^3$)	Pb-PM_{10} ($\mu g/m^3$)	Pb-$PM_{2.5}$ ($\mu g/m^3$)	Pb-PM_1 ($\mu g/m^3$)	$\frac{\text{Pb-PM}_{10}}{\text{Pb-TSP}}$	$\frac{\text{Pb-PM}_{2.5}}{\text{Pb-TSP}}$	$\frac{\text{Pb-PM}_{1}}{\text{Pb-TSP}}$	$\frac{\text{Pb-PM}_{2.5}}{\text{Pb-PM}_{10}}$
Singh et al. (2002)	Traffic + Industrial	–	0.0069	0.0059	0.0051	–	–	0.67[c]	0.86
	Receptor	–	0.0039	0.0021	0.0017	–	–	0.41[c]	0.60
Harrison et al. (2003)	9 m from a highway	–	0.0274	–	–	0.98	0.89	0.80	0.90
Lough et al. (2005)	Traffic Tunnel	–	–	–	–	0.85[d]	0.39[d]	0.20[d]	0.46[d]
		–	–	–	–	–	–	–	0.17[e]
Zereini et al. (2005)	Main street	0.0326[f]	–	–	–	–	–	0.45[c]	0.59
	Side street	0.0126[f]	–	–	–	–	–	0.60[c]	0.78
	Rural	0.0116[f]	–	–	–	–	–	0.64[c]	0.82
Goforth and Christoforou (2006)	Rural	0.0150	–	0.0061	–	–	0.41	–	–
Sabin et al. (2006b)	10 m from hwy	0.0200	0.0132	–	–	0.66	–	–	–
	Urban bkg	0.0110	0.0091	–	–	0.83	–	–	–
Wang et al. (2006d)[a]	Traffic + Industrial	0.0045	0.0044	0.0031	0.0017	0.99	0.69	0.37	0.69
Dall'Osto et al. (2008)[a,g]	Near a large steelwork site + major motorway	0.0306	0.0290	0.0245	0.0140	0.95	0.80	0.46	0.84
Brüggemann et al. (2009)	Curbside of a busy street	–	0.0169	0.0154	0.0120	–	–	0.71[c]	0.86

Table 2-9 (Continued): Summary of studies reporting Pb size distribution in the peer-reviewed literature.

Study	Location or Site Type	Pb-TSP ($\mu g/m^3$)	Pb-PM_{10} ($\mu g/m^3$)	Pb-$PM_{2.5}$ ($\mu g/m^3$)	Pb-PM_1 ($\mu g/m^3$)	Pb-PM_{10} / Pb-TSP	Pb-$PM_{2.5}$ / Pb-TSP	Pb-PM_1 / Pb-TSP	Pb-$PM_{2.5}$ / Pb-PM_{10}
Zota et al. (2009)	Near mine waste	–	0.0114	0.0035	–	–	–	–	0.31
	Traffic + Mine waste	–	0.0052	0.0022	–	–	–	–	0.42
	Upwind	–	0.0030	0.0019	–	–	–	–	0.63
Makkonen et al. (2010)	Rural bkg								
	No wildfire	–	0.0099	0.0055	0.0035	–	–	0.35[c]	0.55
	Wildfire	–	0.0153	–	0.0097	–	–	0.64[c]	–

[a]TSP calculated as a sum of all size fractions.

[b]PM >0.43 µm.

[c]PM_1:PM_{10}.

[d]Estimated from mass emissions distribution measured using MOUDIs.

[e]University of Wisconsin samplers.

[f]PM >0.22 µm.

[g]0.10 µm <PM <75 µm.

[h]Values converted from ng/m^3 to $\mu g/m^3$, a correction to Table 3 presented in Cho et al. (2011).

Source: Adapted from Cho et al. (2011)

Airborne Pb near Metals Industries

Differences among size distributions have been noted for studies contrasting industrial and background sites. Yi et al. (2006) collected Pb-PM size distribution in an industrial area of Jersey City, NJ and contrasted it with the Pb-PM size distribution in suburban New Brunswick, NJ, which is influenced only by traffic. Yi et al. (2006) sampled size distribution for Pb-bearing particles with a MOUDI (cut point range: 0.18-18 µm) along with a coarse particle rotary impactor (CPRI) collecting particles ranging in size from 14.4-100 µm. In the industrial area, 27% of Pb-PM were larger than PM_{10} (avg. Pb-TSP: 9.7 ng/m^3), while in the suburban area 7% of Pb-PM were larger than PM_{10} (avg. Pb-TSP: 6.6 ng/m^3). Singh et al. (2002) used a MOUDI to measure the mass distribution of Pb-PM_{10} in the coarse and fine PM size ranges (cut points range: 0.10-10 µm) for the Downey site along the Alameda industrial corridor in Los Angeles, CA and a site approximately 90 km downwind in Riverside, CA. At the industrial site, the Pb-PM_{10} size distribution was unimodal with a concentration peak in the 100-350 nm size range. The sum of the geometric mean concentrations in each size bin was 13 ng/m^3 for the Downey data. At the downwind site, a bimodal distribution was observed with peaks in the 2.5-10 µm bin and the 350 nm-1 µm bin. The sum of the geometric mean concentrations in each size bin was 7 ng/m^3 for the Riverside data. The authors suggested that higher wind speeds in Riverside compared with the Downey site are effective in resuspending larger particles from the ground to create a peak in the coarse mode of the distribution.

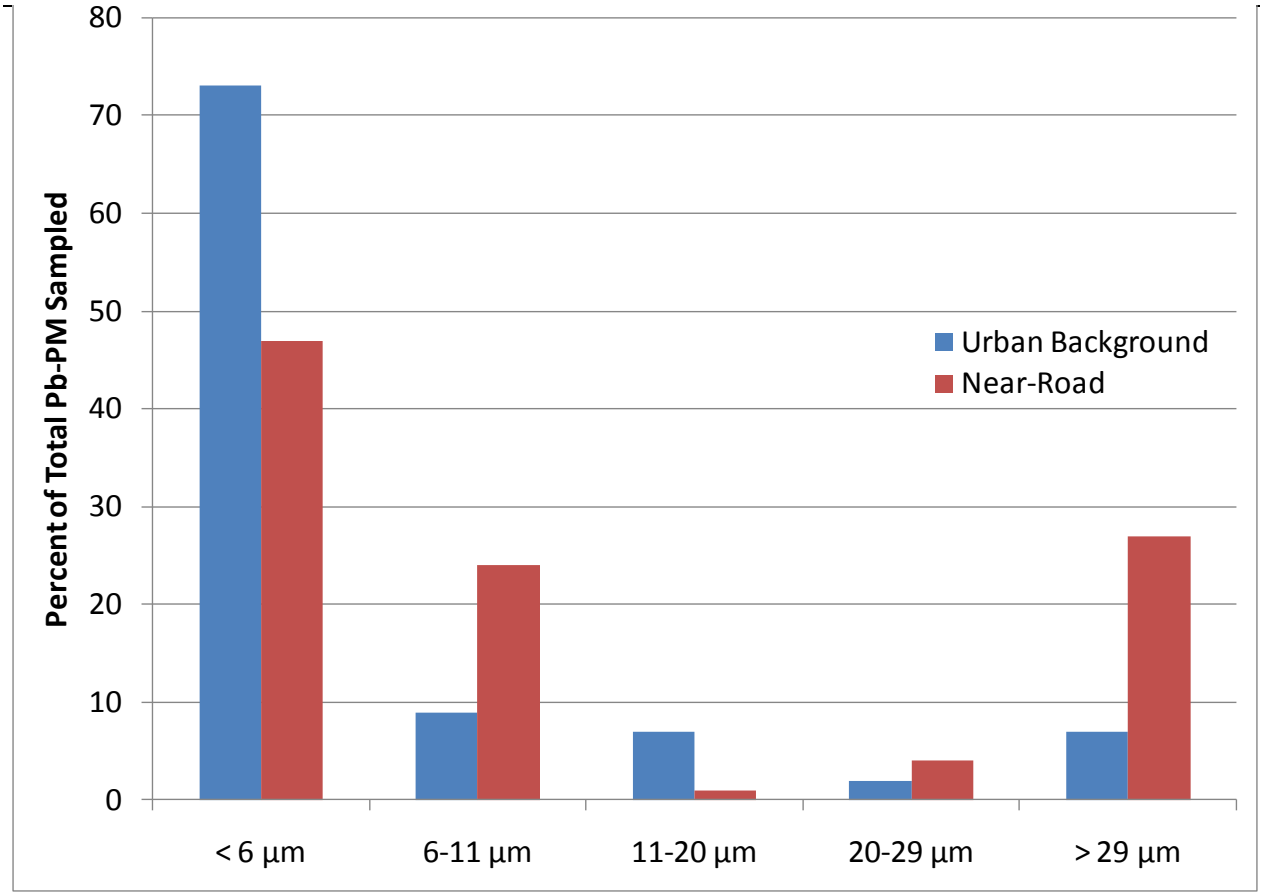
Recent studies have indicated temporal variation in the size distribution given differences among wind direction and industrial production. Bein et al. (2006) measured the size distribution of PM containing Pb from the Pittsburgh Supersite using rapid single particle mass spectrometry and a MOUDI. Source apportionment illustrated that Pb was contained in a sub-population of particles of almost every major particle-containing class in this study, emanating from point sources including fuel combustion, steel processing, incinerators, foundries, battery manufacturing, and glass manufacturing (Pekney et al., 2006). Bein et al.'s (2006) measurements yielded different results on different days, with a bimodal distribution with modes around 140 nm and 750 nm during an October, 2001 measurement and a single dominant mode around 800 nm during a March, 2002 measurement. Differences in the size distributions could have been related to differences among wind speed, wind direction, and source contributions on the respective dates. Weitkamp et al. (2005) used a HI-VOL sampler to measure Pb-bearing $PM_{2.5}$ concentrations across the river from a coke plant in the Pittsburgh, PA area and analyzed the data with ICP-MS. Pb comprised 0.088% of the $PM_{2.5}$ mass, and the mode of the size distribution (measured overall but not specifically for Pb) was observed to shift between

50 nm to 1 μm. Dall'Osto et al. (2008) used a MOUDI (cut points range: 0.196-18 μm) to measure the size distribution of Pb emissions from a steel works facility in a coastal town within the United Kingdom (U.K.). The size distribution was multimodal with a primary mode around 1 μm, a secondary mode around 300 nm, and a very small additional mode around 5 μm. This multimodal distribution was thought to be associated with sintering and steel working processes, from which Pb was emitted. Pekey et al. (2010) measured $PM_{2.5}$ and PM_{10} concentrations in a heavily industrialized area of Kocaeli City, Turkey using a low-volume PM_{10} stack filter unit. They observed $PM_{2.5}/PM_{10}$ ratios of 0.60 during summer and 0.45 during winter.

Airborne Pb Near Roadways

Traffic-induced turbulence may be a cause of resuspension of Pb-bearing particles from deposited contemporaneous wheel weights, industrial emissions, or historic sources. Pb mass in near-road PM is predominantly associated with the coarse mode (U.S. EPA, 2006b). The Pb fraction in resuspended dust generally ranges from 0.002 to 0.3%, with the highest fractions observed for paved road dust and lowest for agricultural soil. Sabin et al. (2006b) compared the size distribution of coarse Pb-PM measured using a Noll Rotary Impactor at an urban background site and at a location 10 meters from the I-405 Freeway in the southern California air basin; data from Sabin et al. (2006b) are displayed in Figure 2-23. For both the urban background and near-road sites, the largest fraction was from PM sampled below the 6 μm cut point, but the near-road Pb-PM distribution appeared bimodal with a mode in the largest size fraction. Sabin et al. (2006b) point out that the freeway tends to be a source of very large particles that are dispersed via the turbulent motion of the vehicular traffic. Song and Gao (2011) used an eight-stage MOUDI (cut point range: 0.18-18 μm) to measure roadside PM, 5 meters from the New Jersey Turnpike in Carlstadt, NJ and speciated the samples. They observed a bimodal distribution of the Pb concentration in summer and a trimodal distribution in winter. 85% of the Pb-PM mass was measured as $PM_{2.5}$ during the summer, and 68% was measured as $PM_{2.5}$ in the winter. Similarly, Zereini et al. (2005) observed that roughly 80% of particle-bound Pb measured with a MOUDI was smaller than 5.8 μm for an urban main street, and more than 90% were smaller than 5.8 μm for a rural area included in that study. However, in a study of automotive emissions in a traffic tunnel, Lough et al. (2005) observed that 85% of Pb measured with a MOUDI was in the PM_{10}, with just 39% in the $PM_{2.5}$ fraction and 20% in the PM_1 fraction. In a near-road study conducted in Raleigh, NC with a 13-stage low-pressure impactor, Hays et al. (2011) note that the proportion of Pb within particles in ultrafine, fine, and coarse size ranges was the same at 50 mg/kg; similar to Lough et al. (2005), mass concentrations were measured by Hays et al. (2011) to be 0.4 ± 0.4 ng/m^3, 1.4 ± 0.6 ng/m^3, and 0.1 ± 0.02 ng/m^3 for $PM_{10-2.5}$,

$PM_{2.5-0.1}$, and $PM_{0.1}$, respectively. The Pb-PM_{10} samples from Hays et al. (2011) were highly correlated with As samples ($\rho = 0.7$, $p < 0.0001$); both Pb and As are found in wheel weights (see Section 2.2.2.6). Hays et al. (2011) did not report correlations between Pb and As for smaller size fractions, but they did state that the correlations for other size fractions were lower compared with Pb-PM_{10} and As. Likewise, the Pb samples were not well correlated with crustal elements in the coarse size distribution, so it is more likely that resuspended Pb originated from contemporary roadway sources rather than historic Pb on-road gasoline emissions. Chen et al. (2010b) measured Pb in $PM_{10-2.5}$, $PM_{2.5-0.1}$, and $PM_{0.1}$ using a MOUDI at a roadside location and in a tunnel in Taipei, Taiwan in 2008. While roadside and tunnel concentrations of PM_{10} and $PM_{2.5}$ were roughly equivalent around 20-30 ng/m^3, Pb in $PM_{0.1}$ was approximately 15 times higher in the tunnel (during the hours 9:00 a.m. – 9:00 p.m.) than by the roadside (tunnel: 20 ng/m^3; roadside: 1 ng/m^3). The authors suggest that particle-bound Pb was emitted from on-road gasoline and diesel engines. This could possibly be attributed to trace levels of Pb in diesel fuel and lubricating oil. Birmili et al. (2006) compared concentrations of Pb in PM measured with a Sierra-Anderson high volume cascade impactor at various traffic and background sites in Birmingham, U.K. Birmili et al. (2006) captured PM at the stage below a 0.5 µm cutpoint and on the 1.5-3.0 µm stage for near-road, in a traffic tunnel, and remote and urban background sites. The highest concentrations were measured in the tunnel, at 3.3 ng/m^3 for Pb-$PM_{0.5}$ and 10 ng/m^3 for Pb-$PM_{1.5-3.0}$. In contrast, urban background was more enriched in the finer size fraction, with concentrations of 5.4 ng/m^3 for Pb-$PM_{0.5}$ and 0.84 ng/m^3 for Pb-$PM_{1.5-3.0}$. Remote background concentrations were on 0.16 ng/m^3 for Pb-$PM_{0.5}$ and 0.03 ng/m^3 for Pb-$PM_{1.5-3.0}$. Bruggemann et al. (2009) measured roadside distribution of Pb in PM in Dresden, Germany using a 5-stage Berner-type low-pressure impactor to analyze the effect of season and direction of the air mass. For all data combined as well as for data broken down by season or by wind direction, it was found that the data followed a unimodal distribution with a peak at the 0.42-1.0 µm size bin with roadside measurements averaging 13-22 ng/m^3, depending on wind direction. Evidence of Pb in road dust related to near road ambient air Pb concentrations is described in Section 2.6.1



Source: Adapted, with permission of Elsevier Publishing, Sabin et al. (2006b).

Figure 2-23 Comparison of urban background and near-road size fractions of Pb-bearing PM.

Several studies have suggested that near-road ambient air Pb samples are derived from sources other than from the road. Harrison et al. (2003) measured the distribution of Pb in PM_{10} at a roadside sampler in Birmingham, U.K. using a MOUDI fitted with only stages 1, 2, 4, and 8 with cutpoints of 10 μm, 2 μm, 1 μm, and 0.2 μm. The size distribution was unimodal with approximately 2% of the Pb mass (totaling 26.5 ng/m^3) above the 10 μm cut point, 12% of the mass in the 2-10 μm bin, 8% in the 1-2 μm bin, 53% of the Pb mass in the 0.2-1 μm bin, and 25% collected below the 0.2 μm cut point. Regression analysis against NO_X concentration in the Harrison et al. (2003) paper provided a weak indication that Pb-$PM_{0.2}$ was associated with NO_X ($\beta = 0.067$, $R^2 = 0.38$) as well as PM_{10} ($\beta = 0.26$, $R^2 = 0.35$). Brüggemann et al. (2009) observed a unimodal Pb size distribution with 51% of the mass in the 0.42-1.2 μm size bin. Observed Pb-PM_{10} concentration was 17 ng/m^3. During winter, Pb concentrations were more than twice as high as during the summer (winter: 24 ng/m^3; summer: 10 ng/m^3), and they were also higher when winds blew from the east (0.42-1.2 μm mode, east: 60 ng/m^3; west: 25 ng/m^3). Brüggemann et al. (2009) suggested that this finding reflected coal burning sources dominating Pb emissions rather than road dust resuspension during winter. Wang et al. (2006d) used a nine-stage cascade impactor (cut point range: 0.43-11 μm) to measure the Pb-PM size distribution in a heavily trafficked area of Kanazawa, Japan with incineration and generation facilities nearby. They observed a bimodal distribution with modes at the 0.65-1.1 μm and the 3.3-4.7 μm size bins. Average concentration in the coarse mode was 2.1 ng/m^3, while fine-mode average concentration was 3.7 ng/m^3. Wang et al.'s (2006d) source apportionment work in this study suggested that the fine mode derives from incineration and combustion of oil and coal.

Airborne Pb at Other Urban and Rural Sites

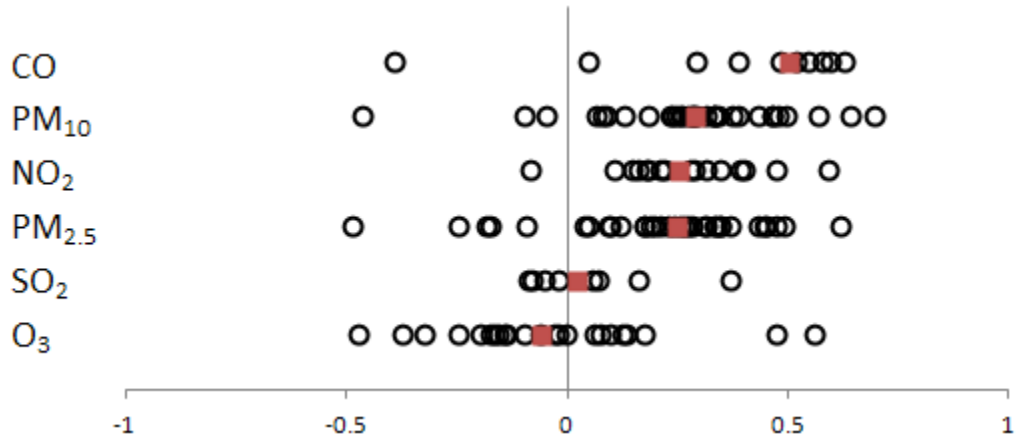
Spatial and temporal concentration variability is also reflected in varying Pb-PM size distributions within and between cities. Martuzevicius et al. (2004) measured the size distribution of Pb in Cincinnati, OH at the city center site using a MOUDI and showed it to be bimodal with a primary peak at 0.56 μm and a slightly smaller secondary peak at 5.6 μm. Using high volume samplers, Moreno et al. (2008) measured Pb concentrations in $PM_{2.5}$ and PM_{10} at urban, suburban, and rural sites around Mexico City, Mexico to illustrate differences among the land use categories. At the urban site, $PM_{2.5}/PM_{10}$ ratios were 0.51 during the day and 0.57 at night (Pb-PM_{10} was 59 ng/m^3 and 162 ng/m^3, respectively). At the suburban site, Pb-$PM_{2.5}$/Pb-PM_{10} ratios were 0.63 during the day and 0.81 at night (Pb-PM_{10} was 24 ng/m^3 and 42 ng/m^3, respectively). Goforth and Christoforou (2006) measured Pb-TSP and Pb-$PM_{2.5}$ with a high volume cyclone separator in rural Georgia and observed a Pb-$PM_{2.5}$ concentration of 6 ng/m^3 and a

Pb-TSP concentration of 15 ng/m^3. Makkonen et al. (2010) measured concentrations of Pb-PM_1, Pb-$PM_{2.5}$, and Pb-PM_{10} during a spate of wildfires in rural southeastern Finland with high volume size-selective samplers. They found that the ratio of Pb-PM_1/Pb-PM_{10} varied substantially from day to day (examples provided of 64% on 8/14/07 and 35% on 8/25/07, with Pb-$PM_{2.5}$/Pb-PM_{10} ratio of 51% on 8/25/07), and they attributed the highest concentrations to long-range transport of wildfire emissions via southerly winds; variability in concentration and ratios was related to shifting wind conditions.

2.5.4 Pb Concentrations in a Multipollutant Context

The correlations between Pb and copollutant concentrations were investigated because correlation may indicate commonality of sources among the pollutants. For example, correlation between Pb and SO_2 may suggest common industrial sources. Correlation between Pb and NO_2 or CO may suggest roadway sources, such as trace Pb in unleaded on-road gasoline or resuspension of material from pulverized wheel weights or contaminated soil. Additionally, seasonality can influence correlations, potentially from differences among sources or the contaminants' responses to climate differences.

Pb concentrations exhibit varying degrees of association with other criteria pollutant concentrations. At most sites, Pb monitors are co-located with monitors for other criteria pollutants, but monitoring the full suite of criteria pollutants at a single monitoring site is rare. As a result, the number of observations for each copollutant varies. Pearson correlations of monitored non-source Pb-TSP concentrations with concentrations of other criteria pollutants are summarized in Figure 2-24 for 2008-2010 data for 46 Pb-TSP monitors at which data were above MDL and more than 30 data pairs were measured at each point. Seasonal co-pollutant measurement data from the literature are also provided in the Chapter 2 Appendix (see Figure 2-63 through Figure 2-67).



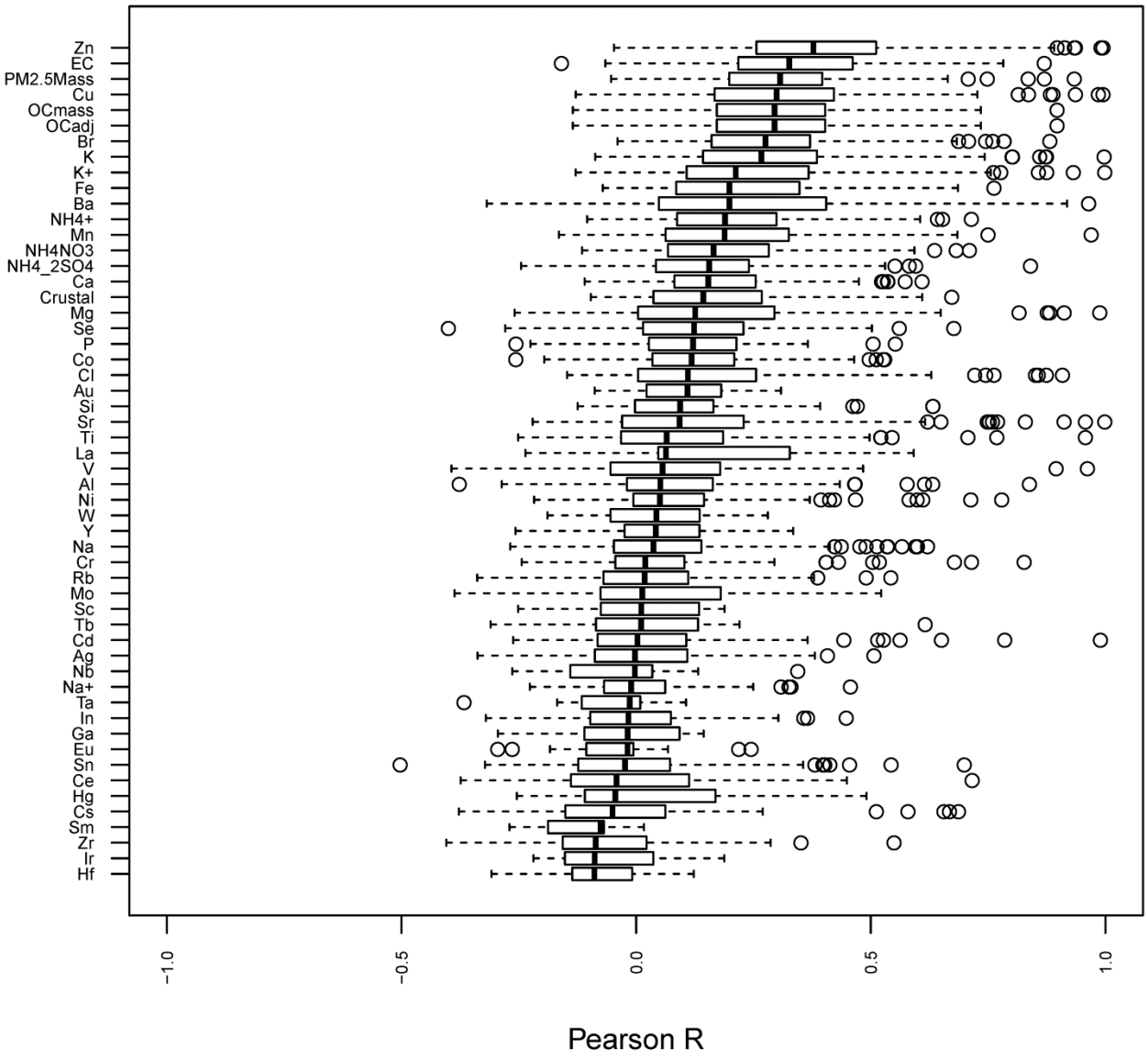
Note: Correlations were calculated from available data when data were above MDL and there were at least 30 data pairs available for comparison. Number of data pairs for each pollutant varies as follows: CO: 10, PM_{10}: 34, $PM_{2.5}$: 34, SO_2: 9, O_3, MDL for Pb varied with sampling conditions; median MDL = 0.0075 µg/m^3. MDL: O_3: 5 ppb, SO_2: 2 ppb, NO_2: 5 ppb, CO: 0.5 ppm, PM_{10}: 4 µg/m^3, $PM_{2.5}$: 2 µg/m^3.

Correlations for individual sites are shown with black open circles, while median correlations are illustrated with a red square.

Figure 2-24 Pearson correlations of monitored non-source daily average Pb-TSP concentration with daily averages of copollutant concentrations, 2008-2010.

Overall, non-source Pb-TSP was most strongly associated with CO (median R = 0.51), with positive Pearson correlation coefficients observed at all but one site, followed by $PM_{2.5}$, and PM_{10} (median R = 0.31 and 0.28, respectively). The relatively high correlations between Pb-TSP and CO may suggest common combustion sources affecting the pollutants. Mobile sources are the largest contributors of CO emissions (U.S. EPA, 2010). However, given that Pb emissions are no longer from tailpipes, correlations may be related to other on-road Pb emissions, such as tire weights. Alternately, the higher correlation with CO could reflect coincident levels of traffic and occurrence of Pb emissions from various sources such as fossil fuel combustion or piston-engine aircraft emissions. Overall correlation coefficients between Pb and NO_2 and between Pb and SO_2 were also positive at most sites, but associations were generally weaker (median R = 0.22 for NO_2, 0.10 for SO_2). The poorest associations were observed between Pb and O_3 (median ρ = -0.04). Spearman correlations, illustrated in Figure 2-62 in the Chapter 2 Appendix, were similar in magnitude and direction with the exception of $PM_{2.5}$, which had a slightly lower median Spearman correlation than both PM_{10} and NO_2.

The relationship between Pb and other species in $PM_{2.5}$ is explored in Figure 2-25, which describes data from 2008-2010 CSN results using Pearson R. These data provide a national perspective on relationships between the various bulk and elemental species monitored in the CSN network. Correlations were only obtained when the data were above MDL and at least 30 data pairs were available.



Note: Correlations were calculated from available data when data were above MDL and there were at least 30 data pairs available for comparison; organic carbon (OC) samples were blank-adjusted.

Figure 2-25 Pearson correlations of monitored Pb-$PM_{2.5}$ concentration with copollutant concentrations, 2008-2010.

Associations between Pb-$PM_{2.5}$ and other species were generally low-to-moderate. The strongest association was with zinc (Zn) (median R = 0.38). Elemental carbon (EC), $PM_{2.5}$ mass, copper (Cu), organic carbon (OC) mass, bromine (Br), and potassium (K) also exhibited low-to-moderate associations with Pb-$PM_{2.5}$ concentrations (median R = 0.27 to 0.33). Such correlations may suggest some common sources affecting the pollutants, as described in Section 2.2.2. For example, correlation with EC and OC mass may be diagnostic indicators of some crustal, general combustion, wood burning, industrial emission, and coal combustion processes. Piston-engine aircraft emit Pb as $PbBr_2$ so this source may explain the weak covariation in Pb and Br concentrations at the CSN sites. At the same time, these species must have other disparate sources that drive the Pearson correlations down.

A few recent studies have used speciation techniques to characterize Pb and other components of PM_{10}, $PM_{2.5}$, and PM_1. Pingitore et al. (2009) used XAFS to speciate air samples obtained near a defunct smelter in El Paso, TX, in 1999 and 2005 and found that air Pb-TSP concentrations of 0.10 to 0.50 µg/m^3 could largely be attributed to Pb-humate. Similarly, Laidlaw et al. (2012) observed statistically significant associations between ambient air Pb-$PM_{2.5}$ and ambient air soil in Pittsburgh, PA, Chicago, IL, Detroit, MI, and Birmingham, AL (R^2 = 0.31-0.49, p <0.01). Together, these results suggest a link between soil resuspension and Pb-TSP levels

Murphy et al. (2008) studied weekly patterns of metals and other aerosol components using data collected from 2000 to 2006 at IMPROVE sites. The authors concluded that Pb concentrations were impacted by piston aircraft emissions. They reached this conclusion because, in contrast to other species, Pb was elevated on weekends when there is typically a peak in general aviation flights. The authors also note that Zn and Pb were highly correlated in atmospheric samples, and they suggest that this is due to similar sources (i.e., electric utility and industrial sources). Murphy et al. (2007) also carried out a detailed study of the distribution of Pb in single atmospheric particles. During the fifth Cloud and Aerosol Characterization Experiment in the Free Troposphere (CLACE 5) campaign conducted at the Jungfraujoch High Altitude Research Station, Switzerland, about 5% of analyzed aerosol particles in PM_1 contained Pb. Of these, 35% had a relative signal for Pb greater than 5% of the total mass spectrum measured by an aerosol time of flight mass spectrometer (ATOFMS). These “high Pb” particles also contained one or more positive ions (e.g., of Na^+, Mg^{2+}, Al^{3+}, K^+, Fe^{3+}, Zn^{2+}, Mo^{6+}, Ag^+, Ba^{2+}). Sulfate fragments were present in 99% of the negative ion spectra associated with high Pb particles, and 50% also contained nitrite and nitrate. About 80% contained positive and/or negative polarity organic fragments. The average aerodynamic diameter of the Pb-rich particles (500 nm) was larger than the background aerosol (350 nm) but none had a diameter less than 300 nm. Murphy et al. (2007) suggest that this mixture can be

attributed to combined emissions from combustion (e.g., Pb and organics) and industry (e.g., Pb sulfates).

2.5.5 Background Pb Concentrations

The 2006 and 1986 Pb AQCDs evaluated evidence on Pb emissions from natural sources, such as volcanoes, sea-salt spray, biogenic sources, wild forest fires and wind-borne soil particles in rural areas without elevated Pb soil concentration. The 1986 Pb AQCD concluded that the natural particulate Pb concentration was probably lower than the concentration of 0.076 ng/m^3 reported at the South Pole and estimated 0.05 ng/m^3 to be the natural background concentration (U.S. EPA, 1986a). A 1980 National Academy of Sciences (NAS) report estimated that average natural background levels of airborne Pb might range from 0.02 to 0.5 ng/m^3 (NAS, 1980).

Global transport can carry airborne Pb to remote areas with no industrial activity, thus it is difficult to estimate a natural background concentration of Pb. Hong et al. (1994) found that Pb concentrations in Greenland ice cores remained nearly constant (at about 0.55 pg Pb/g ice) from about 7,760 years ago to about 3,000 years ago. Ratios of Pb to major crustal elements were not enriched compared with natural background levels in this section of the ice core suggesting that Pb was natural in origin, produced by rock and soil dust. At about 2,500 years ago, Pb concentrations started to increase (to about 100 pg Pb/g snow averaged from 1930 to 1990) (Boutron et al., 1991) corresponding to an enrichment of ~200 times natural background levels. McConnell and Edwards (2008) also noted elevated Pb levels in Greenland ice cores, with high correlation to black carbon (BC), cadmium (Cd), sulfur (S), and thallium (Tl) during the period 1860-1940, suggesting coal combustion sources from North America. Osterberg et al. (2008) observed elevated Pb levels in a 1970-1998 ice core from Mt. Logan, Canada, indicating elevated Pb levels corresponding to increased industrial activity in Asia during this time period.

Measurements of Pb from IMPROVE sites and source apportionment modeling have been used to assess the potential input from intercontinental transport. Liu et al. (2003) used positive matrix factorization to attribute sources of Asian dust to the measurements at two western IMPROVE sites at high elevations, Crater Lake (Oregon) and Lassen Volcanic Park (California) from 1988 to 2000. Geometric mean Pb concentrations of 0.34 and 0.48 ng/m^3 were found in the samples with only a few percent of these values attributable to transport from Asia. No enrichment in Pb and other metals (As, Cr, Cu, Ni, Pb, V and Zn) above reference Asian-dust material was found. Their results suggest either that arriving air masses did not entrain contributions from Asian pollution sources

or that these contributions were preferentially scrubbed out during transport. Large enrichments in sulfur (S) were found, however, which might have been due to pollution sources but also due to model artifacts. However, other studies have found some evidence of trans-Pacific transport. Murphy et al. (2007) measured single Pb particles off the coast of California (using a National Oceanic and Atmospheric Administration [NOAA] aircraft elevated more than 2 km above ground level). Given the elevation of the measurement and the timing of trans-Pacific plume events, the authors concluded that these Pb-bearing $PM_{2.5}$ originated in Asia. They also noted Pb/Zn ratios in $PM_{2.5}$ at the Mount Zirkel, CO IMPROVE site of 0.6 corresponding to measurements at Mauna Loa, HI in spring, when measurements at other times of year produced Pb/Zn ratios of 0.3-0.4. Ewing et al. (2010) used time series analysis of Pb isotope measurements to estimate Asian and local contributions to Pb-$PM_{2.5}$ concentrations measured at two observatories near San Francisco, CA. They estimated a springtime contribution of Asian dust to Pb-$PM_{2.5}$ measurements. In both the Murphy et al. (2007) and Ewing et al. (2010) studies, the authors conclude that the Asian contribution is still generally less than 1 ng/m^3.

The use of data for $PM_{2.5}$, $PM_{10\text{-}2.5}$, and PM_{10} from monitoring sites in the East will generally result in gross overestimates of background concentrations because anthropogenic sources will cause extensive contamination. Intercontinental transport of African dust contributes to PM and is observed mainly in the Southeast but is apparent on an episodic basis elsewhere in the eastern U.S. [see e.g., 2004 PM AQCD (U.S. EPA, 2004) and 2009 PM ISA (U.S. EPA, 2009a)]. Data obtained at four eastern IMPROVE sites ([1] Moosehorn National Wildlife Refuge, ME; [2] Acadia National Park, ME; [3] Swanquarter, NC; and [4] Cape Romain National Wildlife Refuge, SC) from 2007 to 2009 indicate a median Pb-$PM_{2.5}$ concentration of 1.0 ng/m^3 with a 95th percentile value of 2.5 ng/m^3. As noted above, these sites are likely to be affected by upwind anthropogenic sources within the U.S.

Rough estimates for the natural source of Pb in different size fractions of Pb-PM can be made by multiplying the abundance of Pb in soils by the crustal component of PM in the different size fractions. The mean abundance of Pb in surface rocks is ~20 mg/kg (Potts and Webb, 1992); the 2006 Pb AQCD (U.S. EPA, 2006b) reported Pb concentrations in different types of rocks to range from 3.5 to 32 mg/kg (Reuer and Weiss, 2002). There is substantial variation with location depending on composition, in particular on the abundances of uranium (U) and thorium (Th), since Pb is produced mainly by radioactive decay of these elements. The mean Pb concentration of 863 soil samples taken across the U.S. at 2 meters depth is ~16 mg/kg; this value was derived by sampling residual Pb of the weathered rocks on which they formed [Wedepohl (1978) and references therein].

Concentrations of the Pb content of soils can be used with estimates of the crustal component of $PM_{2.5}$, $PM_{10-2.5}$ (which is mainly crustal), PM_{10}, and TSP produced by wind erosion of natural surfaces to estimate contributions to Pb concentrations in these size fractions. U.S. annual average PM_{10} concentrations in some arid counties most affected by windblown dust in the western U.S. are ~20 $\mu g/m^3$. If it is assumed that these levels of PM_{10} are entirely due to natural wind erosion without any anthropogenic contribution and that the Pb concentration in all airborne size fractions if the same as the Pb concentration in bulk soil or surface rock, an estimate of ~0.3 ng/m^3 for the contribution of wind erosion on natural surfaces to Pb in PM_{10} is obtained; however, it must be observed that the natural contribution is probably lower than this estimate. An assumed ratio 3.5 for TSP to PM_{10} in dust storms, derived by Bacon et al. (2011), indicates a contribution of ~1 ng/m^3 for Pb from natural sources in TSP. The more recent estimate indicates that background airborne Pb concentrations are well below current ambient concentrations. These estimates exceed estimates of natural background presented in the 1986 AQCD (U.S. EPA, 1986a) and the National Academy of Sciences Report (NAS, 1980) by a factor of 2 to 50. Hence, a plausible range of natural background airborne Pb is 0.02 to 1 ng/m^3.

2.6 Ambient Pb Concentrations in Non-Air Media and Biota

There have been some major recent research efforts to characterize geographic and temporal trends in Pb concentrations across a variety of environmental media and biota. In general these concentrations reflect the decreases observed in atmospheric Pb concentrations due to reduced on-road Pb emissions.

The 2006 Pb AQCD (U.S. EPA, 2006b) describes several studies showing higher Pb concentrations in plants grown in Pb contaminated soil related to mine spoils, smelting operations, sludge amendment, contaminated irrigation water, and Pb containing agro-chemicals. In general, metal accumulation occurs more readily for Pb salts applied to soils than for the same quantity of metal in sewage sludge or fly ash. Root uptake is the dominant means of accumulation, and it is strongly influenced by pH. Root vegetables are the most strongly affected, and fruits and grains are the least susceptible. More Pb is also generally found in roots than in other parts of the plant.

The 2006 Pb AQCD (U.S. EPA, 2006b) identified ingestion and water intake as major routes of Pb exposure for aquatic organisms, and it identified food, drinking water, and inhalation as major routes of exposure for livestock and terrestrial wildlife. The 2006 Pb AQCD (U.S. EPA, 2006b) reports data from the U.S. Geologic Service National Water-Quality Assessment (NAWQA), which are updated every ten years. In the

NAWQA survey, maxima concentrations in surface waters, sediments, and fish tissues were 30 µg/L, 12,000 mg/kg, and 23 mg/kg, respectively, compared with median values of 0.50 µg/L, 28 mg/kg, and 0.59 mg/kg. Some of the highest levels of Pb contamination occur near major sources, like smelters, and fatal doses have been measured in tissue from sheep and horses near sources. High levels in cattle have also been observed. Wildlife in urban areas tend to contain higher Pb concentrations than in rural areas, and higher Pb accumulations have been observed for aquatic organisms living in polluted coastal zones than in the open sea. Ingestion of deposited Pb-PM on plant surfaces was consistently observed to be more important than Pb accumulated from soil. Some important variations between animals have been observed, and ruminants appear to be less susceptible to Pb uptake than other animals. Uptake of Pb by lowest trophic levels, including invertebrates, phytoplankton, krill, were described as the most important means of introduction into food chains. Elevated Pb levels have been observed in aquatic organisms that feed from sediments when the sediments contain appreciable Pb. In shrimp, a substantial fraction of Pb can be absorbed from prey, and considerably more accumulated Pb from food has been observed to be irreversibly retained than is the case for dissolved Pb from water. These examples all illustrated that substantial Pb uptake by livestock and wildlife readily occurs in Pb contaminated environments.

2.6.1 Soils

Several studies suggest that soil can act as a reservoir for contemporary and historical Pb emissions. The importance of soil Pb to human exposure is described in Section 3.1. At the same time, soils in remote or rural areas tend to have lower Pb concentrations. The most extensive survey of background soil Pb concentration in the conterminous U.S. was conducted between 1961 and 1976 and comprised 1,319 non-urban, undisturbed sample locations, where 250 cm^3 of soil was collected at a depth of 20 cm (Shacklette and Boerngen, 1984). The lower detection limit was 10 mg/kg, and 14% of the 1,319 samples were below it. The mean Pb concentration was 19.3 mg/kg, the median 15 mg/kg, and the 95th percentile was 50 mg/kg. Sixteen locations had Pb concentrations between 100 and 700 mg/kg. These results were in agreement with 3 previous surveys. When creating the *Ecological Soil Screening Level (Eco-SSL) Guidance and Documents*, the U.S. EPA (U.S. EPA, 2007d, 2003b) augmented these data with observations from an additional 13 studies conducted between 1982 and 1997, most of them limited to one state. The resulting data were summarized using state means for each of the fifty states. Those means ranged between 5 and 38.6 mg/kg, with an overall national mean of 18.9 mg/kg. This is reasonably close to the values reported by Wedepohl (1978) and references therein with a mean soil Pb concentration of roughly 16 mg/kg when samples were taken

at 2-meter depths. Biasioli et al. (2006) contrasted urban and rural soils (tested at soil depths of 0-20 cm) of the same alluvial composition near Torino, Italy to assess the influence of anthropogenic inputs. The urban soils had a median Pb concentration of 117 mg/kg, while the median Pb concentration for rural soil was 19 mg/kg. Table 2-10 presents data from seven metropolitan areas (Cobb et al., 2006). Differences among the intraurban concentration ranges illustrate a high level of spatial variability within individual cities as well as high inter-urban variability. The rural New Orleans site reported relatively low Pb soil concentrations, and the highest average Pb soil concentrations were reported for pre-Katrina samples from the city of New Orleans, although this was not the case for the post-Katrina samples.

Table 2-10 Soil concentrations in various cities, 1992-2005.

City	Avg Pb Concentration (mg/kg)[a]	Pb Concentration Range (mg/kg)[a]
Baltimore, MD		1-10,900
Miami, FL	275	25-1,612
Mt. Pleasant, MI	320	100-840
New Orleans, LA (pre-Katrina)	784	31.7-5,195
New Orleans, LA (post-Katrina)	95.9[b]	9.84-1,880
New Orleans, LA (rural outskirts)	11	4.8-17.3
St. Louis, MO	427	35-1,860
Syracuse, NY	80[b]	20-800[c]

[a]Dry weight basis;
[b]Geometric mean;
[c]Range represents 95% of collected values.
Source: Adapted with permission from the American Chemical Society, (Cobb et al., 2006).

In North American forest soils, Pb concentrations have decreased substantially since the phase out of leaded motor vehicle gasoline. When sampling from the O horizon (often at 0-2 cm), Evans et al. (2005) observed Pb concentrations ranging from 60 to 200 mg/kg in Vermont, Maine, and Quebec, with lower concentrations in Quebec than in southern Vermont in 1979, but in 1996 concentrations had decreased to between 32 and 66 mg/kg with no spatial trend. Johnson and Richter (2010) also observed a substantial decrease in O-horizon (depth not specified) Pb concentrations in soil between 1978 and 2004 in West Virginia, Maryland, Pennsylvania, New Jersey, New York, and Connecticut, with a median change of -65%. However, elevation also appears to be an important factor in determining whether appreciable decreases in Pb concentration have occurred since the phase out of leaded gasoline (Kaste et al., 2006). At sites above 800 meters in the

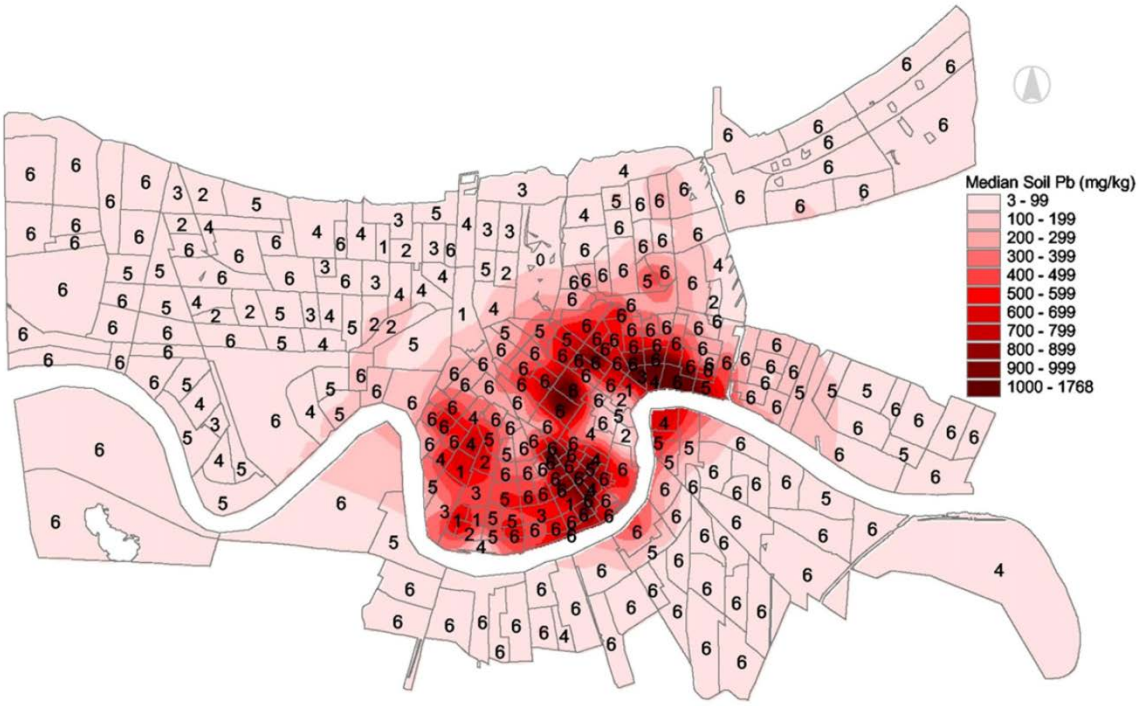
northeastern U.S., O-horizon concentrations (depths not specified) ranged from 11 to 29 kg Pb/ha, and little change in Pb concentration was observed between 1980 and 2000. In contrast, concentrations ranged from 10 to 20 kg Pb/ha at low elevation sites and decreased to 2 to 10 kg Pb/ha by 2000. This difference was likely due to greater organic turnover increasing Pb mobility at the lower elevations (Kaste et al., 2006).

Soil Pb variability depends on the strength and prevalence of nearby sources. Joshi et al. (2009) observed Pb dust concentrations to be highest at industrial sites (260 mg/kg) followed by commercial sites (120 mg/kg) and residential sites (60 mg/kg) in Singapore. Griffith et al. (2002) investigated spatial autocorrelation of soil Pb concentration at three sites: urban Syracuse, NY (0-10 cm), rural Geul River, The Netherlands (0-5 cm), and an abandoned Pb Superfund site in Murray, UT (0-5 cm). In both Syracuse and Geul River, the soil Pb concentrations were not strongly correlated in space, with the exception of soil obtained near roads, which exhibited less variability. The smelting and shooting areas of the Superfund site were both demonstrated to have spatial clusters that were well correlated. Later work on the spatial distribution of metals in Syracuse (sampling depth not specified) produced similar results for that city (Griffith et al., 2009). These studies did not adjust for age of housing, although Griffith et al. (2009) did find that housing age and Pb co-vary. An association between housing age and soil Pb would likely be enhanced by such co-variation.

Emissions trends have shown that industrial activities are now one of the largest sources of soil Pb following phase out of Pb in on-road gasoline. Pruvot et al. (2006) compared urban and agricultural soils at depths of 0-25 cm near a closed Pb smelter with soils in similar environments not exposed to smelter emissions in northern France. For samples near the smelter, Pruvot et al. (2006) observed that median soil Pb levels in lawns were roughly 2 times higher, while kitchen garden soil Pb concentrations were 10 times higher and agricultural soil Pb was almost 15 times higher than soil not exposed to smelter emissions. Bonnard and McKone (2009) reported surface soil Pb concentrations at depths of 0-20 cm of 66-493 mg/kg outside homes of children living within 1 km of a Pb smelter in France; air Pb levels reported by Bonnard and McKone (2009) for this town ranged from 0.025-0.20 µg/m^3. The air samples Pingitore et al. (2009) obtained near a defunct El Paso, TX smelter (described in Section 2.5.4) found that the air Pb-TSP concentrations could largely be attributed to Pb-humate, which is created by sorption of Pb onto humic substances in soil and can be resuspended. Spalinger et al. (2007) compared soil Pb samples at depths of 0-2.5 cm from surrounding towns with those from the Bunker Hill Superfund remediation site in Idaho. Median background soil-Pb concentration was 48 mg/kg, while the median soil-Pb concentration at Bunker Hill was 245 mg/kg.

Recent studies of brownfield soils have shown variable Pb concentrations. Van Herwijnen et al. (2007) measured soils at depths of 0-2 cm near a defunct Zn smelter in Avonmouth, U.K. in areas termed low and high contamination by the authors. Total soil Pb concentration in the low contamination area was 315 mg/kg, while soil Pb concentration in the high contamination area was 1,688 mg/kg. Deng and Jennings (2006) tested various Pb extraction methods on soils obtained from over 50 brownfield sites in the greater Cleveland, OH area at depths of 0-5 cm. Comparison of twelve extraction methods for three samples produced a range of 1,780-2,636 mg/kg for one sample, 283-491 mg/kg for a second sample, and 273-499 mg/kg for a third sample. Verstraete and Van Meirvenne (2008) measured Pb in soils at a remediated brownfield site at depths of 0-5 meters in Belgium and reported average Pb concentrations to be 188 mg/kg and 224 mg/kg in two sampling campaigns. Dermont et al. (2010) fractionated soil sampled at depths of 0-150 cm by particle size class and measured the Pb concentration in each. Pb concentrations by size bin were as follows: 125-250 µm: 1,132 mg/kg; 63-125 µm: 1,786 mg/kg; 38-63 µm: 1,712 mg/kg; 20-38 µm: 2,465 mg/kg; 0-20 µm: 3,596 mg/kg. Hence, the highest concentration was in the smallest soil particle fraction. Bulk Pb concentration over 0-250 µm particle sizes was 2,168 mg/kg.

Several studies explore the relationship between soil Pb concentration and land use. Laidlaw and Filippelli (2008) displayed data for Indianapolis, IN showing the Pb concentration at the soil surface (depths not specified) had a smoothed “bull’s eye” pattern. Cities generally have a similar pattern consisting of larger quantities of Pb accumulated within the inner city and smaller quantities of Pb in outer cities (i.e., near the outskirts or suburban areas) (Filippelli and Laidlaw, 2010). Similarly, Filippelli et al. (2005) reported surface (depths not specified) soil Pb concentration distribution to have a maximum at the center of Indianapolis, IN, around the location where two interstate highways intersect, and to decrease with distance away from the center. However, the spatial distribution of Pb was presumed to reflect contributions from historic sources of on-road gasoline (Section 2.2.2.6) and Pb paint (Section 2.2.2.7). In this paper, soil Pb concentrations were also shown to decrease with distance from roadways, but the levels were roughly four times higher in urban areas compared with suburban areas. This is also illustrated for urban scale Pb accumulation in New Orleans, LA during 1998-2000 in Figure 2-26. Brown et al. (2008) also measured soil Pb concentration along three transects of Lubbock, TX at depths of 0-2 cm and observed that soil Pb decreased with increasing distance from the city center, which was the oldest part of the city.



Note: At the urban scale, Pb quantities are largest within the inner-city residential communities that surround the Central Business District where pavement and concrete cover the soil. Note the several orders of magnitude difference between the interior and the exterior areas of the city. Note that the number on each census tract indicates the number of blood Pb samples taken from that tract during the six years from which the study data were obtained.

Source: Reprinted with permission of Elsevier Publishing, Mielke et al. (2007a)

Figure 2-26 Map of median Pb content in soil in New Orleans, 1998-2000.

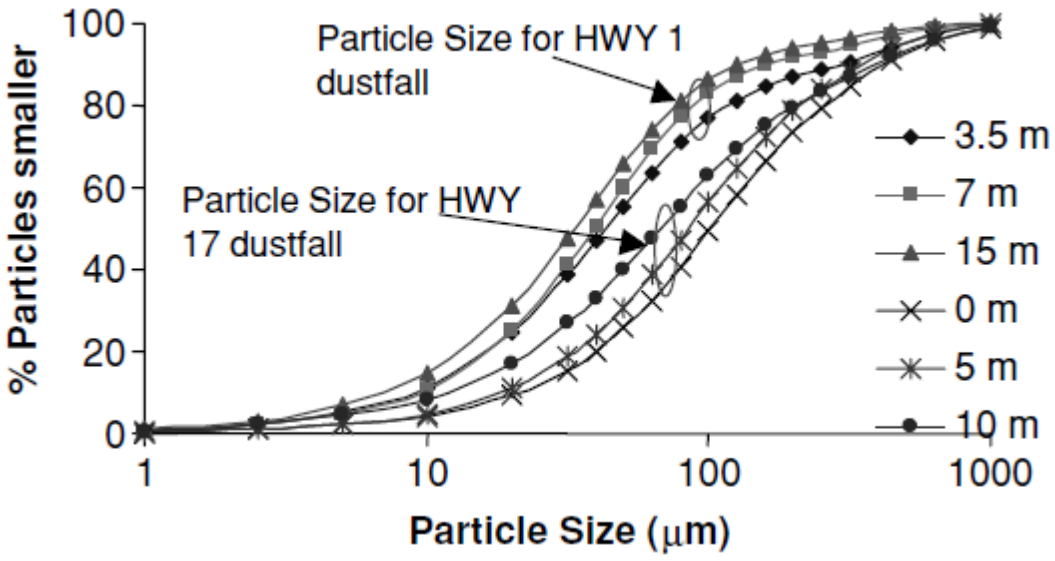
Mielke et al. (2008) compared soil Pb concentrations at depths of 0-2.5 cm for public and private housing at the center and outer sections of New Orleans and found that median and maximum soil Pb concentrations were substantially higher in the city center compared with the outer portions of the city. This study also found that private residences had higher soil Pb compared with public housing. In a separate study to examine surface soil Pb loading and concentration on 25 properties at depths of 0-2.5 cm in 25 New Orleans properties that were previously identified as having median soil Pb concentrations of at least 1,000 mg/kg, Mielke et al. (2007b) reported median and maxima soil loading of roughly 25,000 and 265,000 µg/m^2, respectively. Median and maxima surface soil Pb concentrations were observed to be 1,000 and 20,000 mg/kg, respectively. Clark et al. (2006) performed isotopic analysis on urban garden soils at depths of 0-10 cm and 30-40 cm in an area of Boston, MA with no large industrial sources of Pb and estimated that 40-80% of the soil Pb could be attributed to Pb-based paint while the remainder was attributed to historic Pb on-road gasoline emissions. Additional discussion of historic sources of Pb is provided in Section 2.2.2.7. Isotope

ratios for paint and gasoline references used in the Clark et al. (2006) study were obtained from Rabinowitz (1986).

Several studies have examined the effects of roadway attributes on Pb content in roadside dust. Yesilonis et al. (2008) measured metal content in surface soil samples (0-10 cm) at selected land parcels throughout Baltimore, MD, based on a stratified random sampling design that accounted for land use factors. They compared soil metals within 100 meters buffers of roadways and outside those buffers and found that median soil Pb concentration inside the buffer was significantly higher than median soil Pb concentration outside the buffers (outside: 38.7 kg/ha; inside: 134 kg/ha; $p < 0.0001$). In an analysis of the relationship between land use parameters and Pb concentration in soil in Los Angeles, CA, Wu et al. (2010) observed that soil Pb concentration at depths of 0-2.5 cm was higher near freeways and major traffic arteries compared with other locations. The (square-root transformed) age of the building on a sampled land parcel, length of highway within a 1,000-meter buffer, and length of local road within a 20-meter buffer in which the sample was obtained were significant predictors of Pb. Home age within 30 meters of a soil sample and road length within 3,000 meters of a road sample were also shown to be significant predictors of soil Pb concentration in areas not designated to be near a freeway or major traffic artery. Wu et al. (2010) concluded that both historical traffic and leaded paint contributed to Pb contamination in soils. However, Wu et al. (2010) acknowledged uncertainty in historical roadway and traffic count data, which introduces uncertainty into that conclusion. Study areas were classified as residential, commercial, park, and industrial (not specific to Pb emissions), although the authors were not able to distinguish the relative effects of each area on Pb content in roadside dust. Wu et al. (2010) reported that the highest median measured concentrations of Pb content in roadside dust were in residential freeway samples (112 mg/kg), followed by residential arterial samples (98 mg/kg), and industrial freeway samples (90 mg/kg). Additional sources of Pb to soil near roadways, such as traces of Pb in unleaded gasoline and Pb-containing wheel weights (described in Section 2.2.2.6) were not considered in this study. Amato et al. (2009) observed that deposited PM onto roadways, measured as dust samples, in Barcelona, Spain was differentially enriched with Pb compared with dust collected at a harbor area. Pb concentration in PM_{10} was highest at ring roads (229 mg/kg) and in the city center (225 mg/kg), followed by demolition and construction sites (177 mg/kg) and near a harbor (100 mg/kg). Roadside dust Pb concentration was also found to vary with roadway activity by Preciado and Li (2006); average Pb dust concentrations at a busy road were 90 mg/kg, compared with 56 mg/kg at a less busy road. Preciado and Li (2006) also examined soil Pb depth to ascertain availability of soil Pb for exposure. They observed peak soil Pb concentrations of 250-800 mg/kg at depths of 0.12-0.23 meters, depending on the soil measurement location and roadway traffic.

This finding may suggest that over time, historic emissions of Pb deposited to soil are being covered by fresh soil and hence moving further down within the soil horizons.

Size distributions of Pb-containing dust settled on the ground contain larger particles than the size distribution of ambient air Pb, described in Section 2.5.3. Preciado and Li (2006) measured the size distribution of Pb-containing dust near the roadside, as seen in Figure 2-27. For the busy highway, the mass median dust diameter estimated from the figure ranged from 34- 42 μm, depending on distance from the road. For the lower traffic highway, the mass median dust diameter estimated from the figure ranged from 64-99 μm.



Source: Reprinted with permission of Springer-Verlag Publishing; Preciado and Li (2006)

Figure 2-27 Size distribution of Pb-containing dust collected near busy (HWY 1) and low traffic (HWY 17) highways.

Two recent studies focused on Pb from paint degradation by examining Pb dust loading to hard surfaces located along transects of each of the five boroughs of New York City (Caravanos et al., 2006a; Weiss et al., 2006). Caravanos et al. (2006a) used GIS to examine Pb dust loadings on top of pedestrian traffic signals and observed "hot spots," defined by the authors as at least twice the Pb dust loading at adjacent samples near major elevated bridges in upper Manhattan, the Bronx, and Queens. In Brooklyn and Staten Island, areas with high dust loading were not clearly attributed to a source. "Low spots," defined by the authors as at least two times lower Pb dust loading compared with

adjacent samples were observed in lower Manhattan, were thought to correspond with intensive cleaning efforts that followed the September 11, 2001 World Trade Center attack. Weiss et al. (2006) studied Pb concentrations of grit (granules of mixed composition found to accumulate alongside street curbs) along the transects and found that median Pb concentrations in grit under the elevated steel structures were 2.5-11.5 times higher than those obtained away from steel structures; 90th percentile values were up to 30 times higher near steel structures compared with those further from these structures.

Outdoor Pb dust has been also associated with demolition activities. Farfel et al. (2005, 2003) measured Pb dust within 100 meters of a demolition site before, immediately after, and 1 month following the demolition. They found that the rate of Pb dust fall increased by a factor of more than 40 during demolition (Farfel et al., 2003). Immediately after demolition, one demolition site had dust loadings increase by a factor of 200% for streets (87,000 µg/m^2), 138% for alleys (65,000 µg/m^2), and 26% for sidewalks (23,000 µg/m^2) compared with pre-demolition Pb dust levels. One month following demolition, Pb dust levels dropped by a factor of 45% for the street (48,000 µg/m^2), compared with post-demolition concentrations, 67% for alleys (21,000 µg/m^2), and 41% for sidewalks (14,000 µg/m^2). At another demolition site, smaller increases were observed: 29% for streets (29,000 µg/m^2), 18% for alleys (19,000 µg/m^2) and 18% for sidewalks (22,000 µg/m^2). No values were reported for the 1-month follow-up for the second site (Farfel et al., 2005).

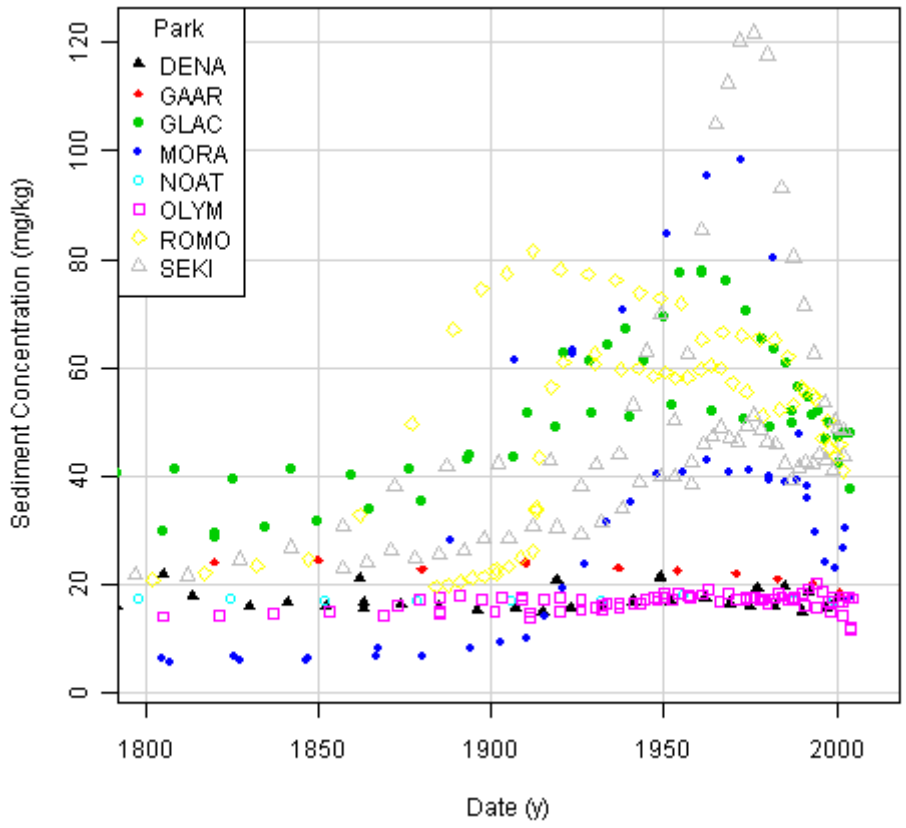
Pb can be elevated in soils located where ammunition is used for military or hunting purposes. In a study of Pb content in sand used to cover a firing range, Lewis et al. (2010) found that 93% of bullet mass was recovered in the top 0.3 meters of the sand, and 6.4% was recovered at a depth of 0.3-0.45 meter. Pb oxides were observed to be the dominant species in the contaminated sand. Berthelot et al. (2008) studied soil Pb concentrations in grounds (0-15 cm) used for testing military tanks and munitions and measured soil Pb levels to range from 250 to 2,000 mg/kg dry basis.

2.6.2 Sediments

The recently completed Western Airborne Contaminants Assessment Project (WACAP) is the most comprehensive database, to date, on contaminant transport and depositional effects on sensitive ecosystems in the U.S. (Landers et al., 2010). The transport, fate, and ecological impacts of semi-volatile compounds and metals from atmospheric sources were assessed on ecosystem components collected from 2002-2007 in watersheds of eight core national parks (Landers et al., 2008). The goals of the study were to assess

where these contaminants were accumulating in remote ecosystems in the western U.S., identify ecological receptors for the pollutants, and to determine the source of the air masses most likely to have transported the contaminants to the parks. Pb was measured in sediments, as well as snow, water, lichen, fish, and moose during the multiyear project, and although Pb was not measured in air as a part of this study, routine monitoring find particle Pb was monitored at IMPROVE sites in the majority of national parks included in the study.

Pb concentrations in sediments from all lakes in which Pb was measured in the conterminous 48 states exhibited higher Pb concentrations near the surface relative to pre-industrial Pb levels measured at greater depth. This was not the case for other metals measured, except for cadmium (Cd) and mercury (Hg). Sediments in most lakes exhibited maximum concentrations between 1960 and 1980, followed by a decrease, as shown in Figure 2-28. A clear decline in Pb concentrations in sediments after the discontinued use of leaded on-road gasoline was observed at almost all WACAP locations, of nearly all WACAP sites in the western U.S. Sediment Pb concentrations averaged over the year in which they were obtained correlated moderately well with annual average Pb-TSP concentrations from the AQS with R = 0.63 for 1980-2004, in which WACAP data were available (NPS, 2011). Pb concentrations in sediments were much lower in Alaska, and no such decline was observed. Pb in sediments was mainly attributed to on-road gasoline use, but for some lakes a strong influence from other local sources of Pb to lake sediments was shown to be important, including Pb mining, smelting, logging, and other industrial activities. The reduction in sediment Pb concentrations shown in Figure 2-28 for recent years coincides with declines in air Pb concentrations following the phase-out of Pb anti-knock agents in gasoline and reductions of air Pb emissions from industrial activities. Elevated Pb deposition at the Glacier, Rocky Mountain, and Sequoia and Kings Canyon National Park and Preserve sites was thought by Landers et al. (2008) to reflect regional scale bioaccumulation of airborne contaminants in remote ecosystems in the western U.S. Accumulation of contaminants was shown to vary geographically; Landers et al. (2008) lists potentially influential factors causing variation in Pb deposition including proximity to individual sources or source areas, primarily agriculture, mining, and smelting operations. This finding was counter to the original working hypothesis that most of the contaminants found in western parks would originate from eastern Europe and Asia.



Note: DENA = Denali (Alaska), GAAR = Gates of the Arctic (Alaska), GLAC = Glacier (Montana), MORA = Mount Rainier (Washington State), NOAT = Noatak (Alaska), OLYM = Olympic (Washington State), ROMO = Rocky Mountain (Colorado), SEKI = Sequoia and Kings Canyon (California).

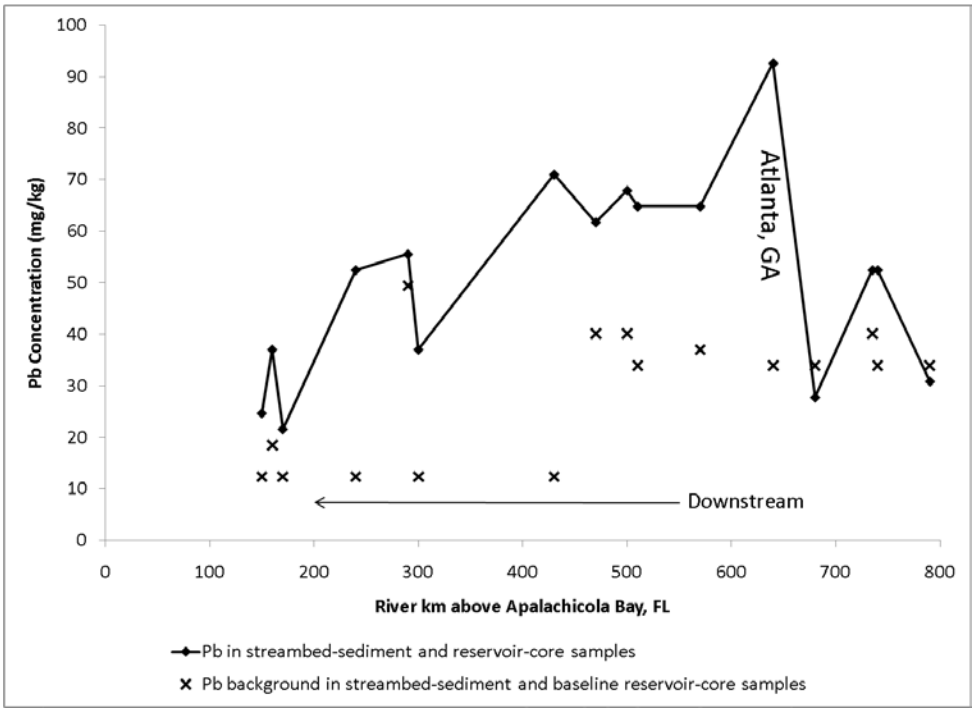
Source: WACAP Database (NPS, 2011)

Figure 2-28 WACAP data for Pb concentration in sediment at eight National Parks and/or Preserves.

In a survey of 35 reservoirs and lakes in 16 continental U.S. states, Van Metre et al. (2006) collected data from sediment cores extending back as far as the early 1800s, and up to 2001. For most locations, they were able to match at least three bodies of water in rural (designated as 'reference'), light urban, and dense urban settings. In reference bodies of water, the median sediment Pb concentration corresponding to the 1990s was 48 mg/kg dry basis. It was 56 mg/kg dry basis in sediments from light urban bodies, and 214 mg/kg dry basis in dense urban ones (Mahler et al., 2006). Using the most distant past sediment records, Mahler et al. (2006) provided approximations of concentrations attributable to anthropogenic inputs. The median of these values for the 1990s were 28, 22, and 194 mg/kg dry basis in the reference, light urban, and dense urban bodies of water, respectively. When examining sediment cores in Lake St. Croix, MN for Pb

trends, Balogh et al. (2010) observed that the mean Pb concentration peaked in the 1970s then declined, with levels from the 1990s below 1930s levels.

Data from select regions of the U.S. illustrate that Pb concentrations in surface waters and sediment are likely to be higher in urbanized areas compared with rural locations. Figure 2-29 illustrates such variability within a single watershed for the Apalachicola, Chattahoochee, and Flint River Basin, which runs south from north of the greater Atlanta, GA metropolitan area and drains into the Gulf of Mexico at the Apalachicola Bay in the Florida panhandle. Sediment concentrations peaked near the Atlanta area and diminished as distance from the Apalachicola Bay decreased. This observation suggests that rural areas have lower Pb sediment levels compared with urban areas. Consistent with the WACAP trends shown in Figure 2-28, the data also illustrated that Pb concentrations in sediment have declined in the U.S. since 1975 (Figure 2-30). Note that Figure 2-30 does not include data near Atlanta, so the urban peak cannot be seen here as in Figure 2-29.

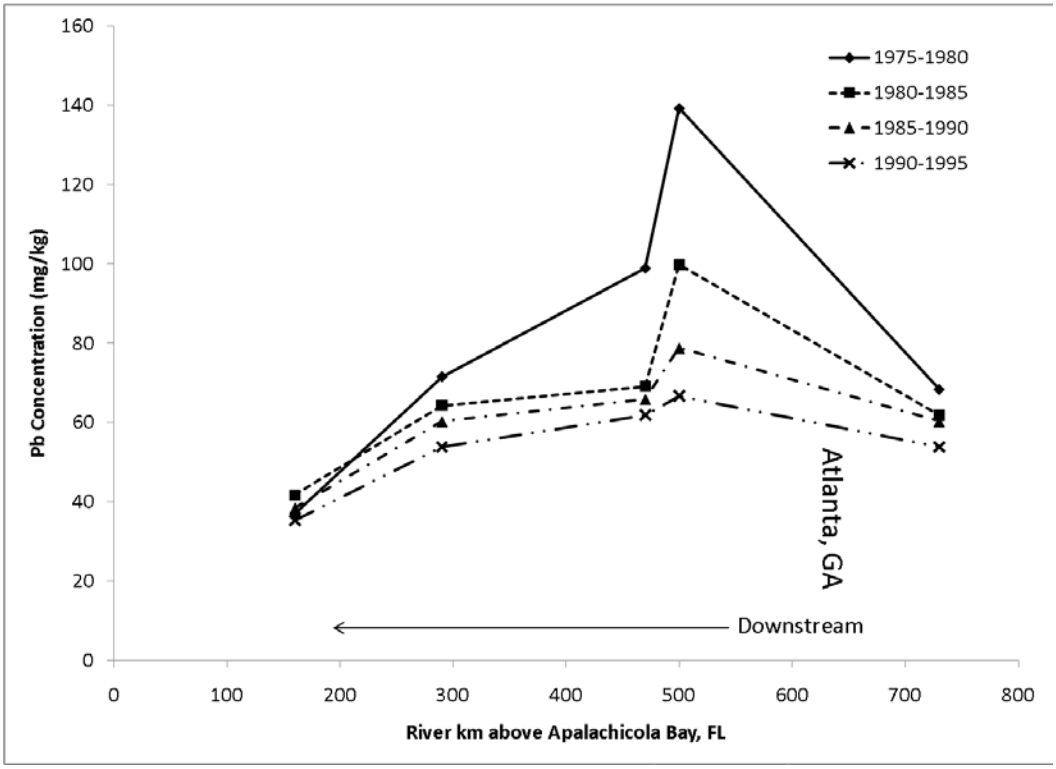


Note: The background refers to concentrations from undeveloped geographic regions and baseline samples are obtained from the bottom of the sediment core to minimize anthropogenic effects on the sample. Pb concentrations reported on a dry basis.

The lakes and reservoirs along the Apalachicola, Chattahoochee, and Flint River Basin (ACF) feed from north of the Atlanta, GA metropolitan area into the Gulf of Mexico at Apalachicola Bay in the Florida panhandle.

Source: Reprinted with permission of the American Chemical Society, Callender and Rice (2000).

Figure 2-29 Sediment core data (1992-1994) for the lakes and reservoirs along the Apalachicola, Chattahoochee, and Flint River Basin (ACF).



Note: The background refers to concentrations from undeveloped geographic regions and baseline samples are obtained from the bottom of the sediment core to minimize anthropogenic effects on the sample. Pb concentrations reported on a dry basis. Sediment samples were not obtained for various time periods in Atlanta, so the graph does not indicate a lack of elevated sediment Pb in Atlanta.

Lakes and reservoirs along the Apalachicola, Chattahoochee, and Flint River Basin (ACF) feed from north of the Atlanta, GA metropolitan area into the Gulf of Mexico at Apalachicola Bay in the Florida panhandle.

Source: Reprinted with permission of the American Chemical Society, Callender and Rice (2000).

Figure 2-30 Sediment core data (1975-1995) for the lakes and reservoirs along the Apalachicola, Chattahoochee, and Flint River Basin (ACF).

Many recent studies have illustrated the effects of natural disasters on Pb concentrations in surface water and sediment in the wake of Hurricane Katrina, which made landfall on August 29, 2005 in New Orleans, LA, and Hurricane Rita, which made landfall west of New Orleans on September 23, 2005. Pardue et al. (2005) sampled floodwaters on September 3 and September 7, 2005 following the hurricanes and observed that elevated concentrations of Pb along with other trace elements and contaminants were not irregular for stormwater but were important because human exposure to the stormwater was more substantial for Hurricane Katrina than for a typical storm. Floodwater samples obtained

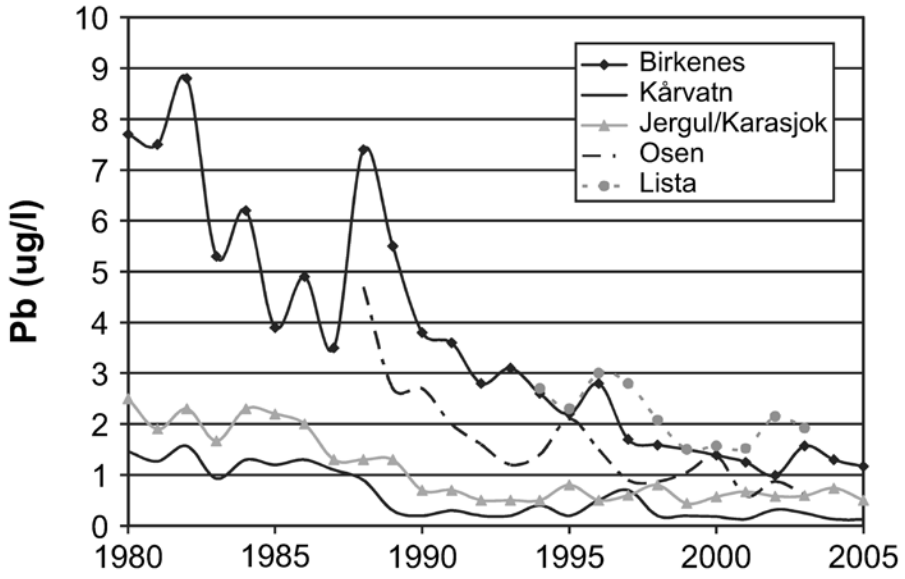
throughout the city on September 18, 2005 and analyzed for Pb by Presley et al. (2006) were below the limit of detection (0.04 µg/mL). Likewise, Hou et al. (2006) measured trace metal concentration in the water column of Lake Pontchartrain and at various locations within New Orleans during the period September 19 through October 9, 2005 and found that almost all Pb concentrations were below the limit of detection (0.0020 mg/kg). However, several studies noted no appreciable increase in Pb concentration within Lake Pontchartrain soils and sediments (Abel et al., 2010; Abel et al., 2007; Schwab et al., 2007; Cobb et al., 2006; Presley et al., 2006). Shi et al. (2010) analyzed Lake Pontchartrain sediment samples using a factored approach and found that most Pb was sequestered in carbonate-rich, Fe oxide-rich, and magnesium (Mg) oxide-rich sediments in which it can be more readily mobilized and potentially more bioaccessible. Zahran et al. (2010) and Presley et al. (2010) noted that soil Pb samples obtained outside schools also tended to decrease in the wake of Hurricanes Katrina and Rita, with some sites observing substantial increases and others noting dramatic reductions. These studies suggest that floodwaters can change the spatial distribution of Pb in soil and sediments to result in increased or reduced concentrations.

2.6.3 Rain

There are currently no routine measurements of Pb in precipitation in the U.S. Recent results from locations outside the U.S. were consistent with decreasing rain water concentrations described in the 2006 Pb AQCD, reflecting the elimination of Pb from on-road gasoline in most countries. From the 2006 Pb AQCD (U.S. EPA, 2006b), volume weighted Pb concentrations in precipitation collected in 1993-94 from Lake Superior, Lake Michigan and Lake Erie ranged from ~0.7 to ~1.1 µg/L (Sweet et al., 1998). These values fit well with the temporal trend reported in Watmough and Dillon (2007), who calculated annual volume-weighted Pb concentrations to be 2.12, 1.17 and 0.58 µg/L for 1989-1990, 1990-1991 and 2002-2003, respectively, in precipitation from a central Ontario, Canada, forested watershed. A similar value of 0.41 µg/L for 2002-03 for Plastic Lake, Ontario, was reported in Landre et al. (2009). For the nearby Kawagama Lake, Shotyk and Krachler (2010) gave Pb concentrations in unfiltered rainwater collected in 2008. For August and September 2008, the values were 0.45 and 0.22 µg/L, respectively, and so there had been little discernible change over the post-2000 period. In support, Pb concentrations in snow pit samples collected in 2005 and 2009 collected 45 km northeast of Kawagama Lake had not changed to any noticeable extent (0.13, 0.17, and 0.28 µg/L in 2005; 0.15 and 0.26 µg/L in 2009) (Shotyk and Krachler, 2010).

There have also been a few recently published, long-term European studies of Pb concentration in precipitation including Berg et al. (2008) and Farmer et al. (2010). Berg

et al. (2008) compared the trends in Pb concentration in precipitation at Norwegian background sites in relation to the decreasing European emissions of Pb over the period 1980-2005. The Birkenes site at the southern tip of Norway is most affected by long-range transport of Pb from mainland Europe but there had been a 97% reduction in the concentration of Pb in precipitation over the 26-year time period. This was similar to the reductions of 95% and 92% found for the more northerly sites, Karvatn and Jergul/Karasjok, respectively (Figure 2-31). A decline of ~95% in Pb concentrations in moss (often used as a biomonitor of Pb pollution) from the southernmost part of Norway, collected every 5 years over the period 1977-2005, agreed well with the Birkenes precipitation results (Berg et al., 2008). The reductions in Pb concentration in both precipitation and moss appear to agree well with the reductions in emissions in Europe (~85%) and Norway (~99%). Similar to the situation in the U.S., the greatest reductions occurred prior to the late 1990s, and relatively minor reductions have occurred thereafter; see Figure 2-31.



Source: Reprinted with permission of Pergamon Press, Berg et al. (2008)

Figure 2-31 Trends in Pb concentration in precipitation from various sites in Norway over the period 1980-2005.

Farmer et al. (2010) showed the trends in concentration of Pb in precipitation collected in a remote part of northeastern Scotland over the period 1989-2007. The 2.6- and 3.0-fold decline in mean concentration from 4.92 µg/L (1989-1991) to 1.88 µg/L (1999) and then to 0.63 µg/L (2006-2007) is qualitatively but not quantitatively in line with the sixfold

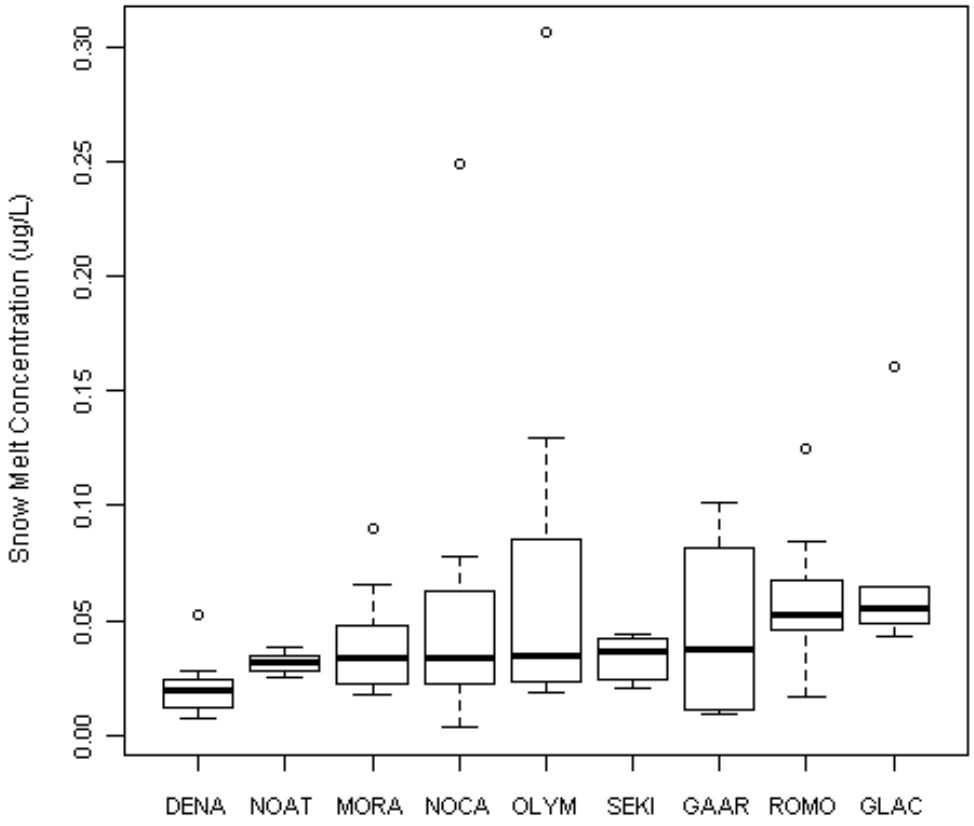
decline in annual total U.K. emissions of Pb to the atmosphere over each of these time periods. After leaded on-road gasoline was banned in the U.K. in 2000, the ratio of rainwater Pb concentrations to Pb emissions (metric tons) appears to have stabilized to a near-constant value of 0.009 µg/L per metric ton. The concentrations in precipitation reported in these studies are all at the lower end of the range reported in the 2006 Pb AQCD (U.S. EPA, 2006b), and similar to concentrations reported for those studies conducted after the removal of Pb from on-road gasoline. Overall, recent studies of wet deposition tended to confirm the conclusions of the 2006 Pb AQCD (U.S. EPA, 2006b) that wet deposition fluxes have greatly decreased since the removal of Pb from on-road gasoline.

2.6.4 Snowpack

The location of Pb deposition impacts its further environmental transport. For example, Pb deposited to some types of soil may be relatively immobile, while Pb deposited to snow is likely to undergo further transport more easily when snow melts. Deposition to snow was investigated in several studies. Measurements of Pb in snowmelt during the WACAP study, showed that median Pb concentration ranged form 20-60 ng/L, with 95th percentile values ranging from 30-130 ng/L; see Figure 2-32 (NPS, 2011). Measurements in WACAP of Hg and particulate carbon deposition onto snow were thought to reflect coal combustion, and Pb was not significantly correlated with Hg in terms of either concentration or of calculated enrichment factors normalized to Al concentrations. Shotyk and Krachler (2009) reported considerably higher concentrations at two North American sites, Johnson and Parnell, in Ontario, Canada. Mean Pb concentration for contemporary snow was 672 (Johnson, n = 6; Parnell, n = 3) ng/L. Shotyk et al. (2010) presented additional values for Pb in contemporary snow samples in Simcoe County, Ontario, and these were higher than for ground and surface waters. Luther Bog and Sifton Bog snow had mean Pb concentrations of 747 and 798 ng/L, respectively. The relatively high concentrations in snow were attributed to contamination with predominantly anthropogenic Pb, although it was noted that the extent of contamination was considerably lower than in past decades.

Seasonal patterns of heavy metal deposition to snow on Lambert Glacier basin, east Antarctica, were determined by Hur et al. (2007). The snow pit samples covered the period from austral spring 1998 to summer 2002 and Pb concentrations ranged from 1.29-9.6 pg/g with a mean value of 4.0 pg/g. This was similar to a mean value of 4.7 pg/g (1965-1986) obtained by Planchon et al. (2003) for Coats Land, northwest Antarctica. Estimated contributions to the Pb in Lambert Glacier basin snow were ~1% from rock and soil dust (based on Al concentrations) and ~4.6% from volcanoes (based on the

concentrations of nss-sulfate). There was almost negligible contribution from seaspray (based on Na concentrations), and so it was suggested that a substantial part of the measured Pb concentration must originate from anthropogenic sources. Highest Pb concentrations were generally observed in spring/summer with an occasional peak in winter. This contrasts with data for the Antarctic Peninsula, where highest concentrations occurred during autumn/winter, and again with Coats Land, where high concentrations were observed throughout the winter. These differences were attributed to spatial changes in input mechanism of Pb aerosols arriving at different sites over Antarctica, which could be due to their different source areas and transport pathways. Hur et al. (2007), however, suggested that the good correlation between Pb and crustal metals in snow samples shows that Pb pollutants and crustal PM are transported and deposited in Lambert Glacier basin snow in a similar manner.



Note: DENA = Denali (Alaska), NOAT = Noatak (Alaska), MORA = Mount Rainier (Washington State), NOCA = North Cascades (Washington State), OLYM = Olympic (Washington State), SEKI = Sequoia and Kings Canyon (California), GAAR = Gates of the Arctic (Alaska), ROMO = Rocky Mountain (Colorado), GLAC = Glacier (Montana).

Source: WACAP Database (NPS, 2011)

Figure 2-32 Box plots illustrating Pb concentration in snow melt at nine National Parks and Preserves.

Lee et al. (2008b) collected 42 snow samples during the period autumn 2004-summer 2005 from a 2.1-meter snow pit at a high-altitude site on the northeast slope of Mount Everest, Himalayas. Pb concentrations ranged from 5-530 pg/g with a mean value of 77 pg/g. The mean value is clearly higher than the Hur et al. (2007) value for Antarctica but is substantially lower than a mean concentration of 573 pg/g for snow from Mont Blanc, France [1990-1991; Lee et al. (2008b)]. The mean Pb concentration for Mount Everest snow was lower during the monsoon (28 pg/g) compared with the non-monsoon periods (137 pg/g). From calculated enrichment factors (Pb/Al_{snow}:Pb/Al_{crust}), anthropogenic inputs of Pb were partly important but soil and rock dust also contributed. The low Pb concentrations during monsoon periods are thought to be attributable to low levels of atmospheric loadings of crustal dusts. Lee et al. (2008b) noted that their conclusions differ from those in Kang et al. (2007), who stated that anthropogenic

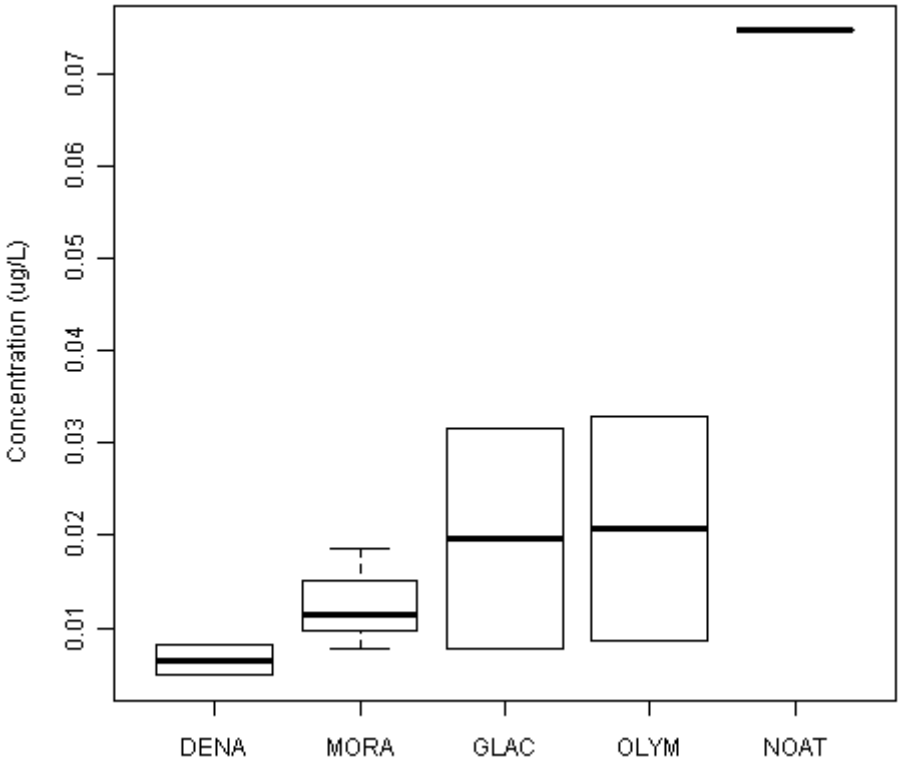
contributions of Pb to Mount Everest snow were negligible because the Everest concentrations were similar to those in Antarctica. Kang et al. (2007) did not take account of the difference in accumulation rates at the two sites and had also used Pb concentrations for Antarctic snow from a study by Ikegawa et al. (1999). Lee et al. (2008b) suggested that these Pb concentrations were much higher than expected and that their snow samples may have suffered from contamination during sampling and analysis.

2.6.5 Natural Waters

Monitoring data for streams, rivers, and lakes are summarized in periodic national assessments of surface waters that are carried out periodically by EPA, and they include measurement of major biological and chemical stressors. Human exposure to Pb in drinking water is described in Section 3.1.3.3. Pb concentrations in natural waters also may reflect deposition of Pb even in remote locations. WACAP data at five National Parks and Preserves show median Pb concentrations in surface waters to range from 6 to 75 ng/L (NPS, 2011); see Figure 2-33. Four sites (Denali, Mt. Ranier, Glacier, and Olympic National Parks) were in the lower range of 6 to 20 ng/L. One site (Noatak) reported a single value of 75 ng/L. With the exception of the Noatak site, the WACAP values were in line with measurements by Shotyk and Krachler (2007) of Pb concentrations in six artesian flows in Simcoe County, near Elmvale, Ontario, Canada. The values ranged from 0.9 to 18 ng/L with a median (n = 18) of 5.1 ng/L. These are comparable with reports of a range of 0.3-8 ng/L for Lake Superior water samples (Field and Sherrell, 2003). Shotyk and Krachler (2007) also commented that such low concentrations for ground and surface waters are not significantly different from those (5.1 ± 1.4 ng/L) reported for Arctic ice from Devon Island, Canada, dating from 4,000-6,000 years ago. In a separate study, Shotyk and Krachler (2009) reported concentrations of Pb in groundwater (from two locations, Johnson and Parnell), surface water (Kawagama Lake [Ontario, Canada]) and contemporary snow (Johnson and Parnell, as described in Section 2.6.4). The lowest mean dissolved Pb concentrations were found for groundwater: 5.9 (Johnson, n = 11) and 3.4 (Parnell, n = 12) ng/L. For lake water the mean Pb concentration was 57 (Kawagama Lake, n = 12) ng/L. The extremely low concentrations of Pb in the groundwaters were attributed to natural removal processes. Specifically, at the sampling location in Canada, there is an abundance of clay minerals with high surface area and high cation exchange capacity and these, combined with the elevated pH values (pH=8.0) resulting from flow through a terrain rich in limestone and dolostone, provide optimal circumstances for the removal of trace elements such as Pb from groundwater. Although such removal mechanisms have not been demonstrated, the vast difference between Pb concentration in snow and that in

the groundwaters, indicate that the removal process is very effective. Shotyk and Krachler (2010) speculate that even at these very low Pb concentrations, much if not most of the Pb is likely to be colloidal, as suggested by the 2006 Pb AQCD (U.S. EPA, 2006b). Finally, Shotyk et al. (2010) suggest that the pristine groundwaters from Simcoe County, Canada, provide a useful reference level against which other water samples can be compared.

Although Pb concentrations in Kawagama Lake (Ontario, Canada) water were approaching "natural values," the $^{206}Pb/^{207}Pb$ ratios for the samples that had the lowest dissolved Pb concentrations of 10, 10 and 6 ng/L were 1.16, 1.15 and 1.16, respectively. These values are inconsistent with those expected for natural Pb (the clay fraction from the lake sediments dating from the pre-industrial period had values of 1.19-1.21) and it was concluded that most of the dissolved Pb in the lake water was of industrial origin. Shotyk and Krachler (2010) found that the full range of isotope ratios for Kawagama Lake water samples (Ontario, Canada) was 1.09 to 1.15; this was not only much lower than the stream water values entering the lake but also lower than the values attributed to leaded on-road gasoline in Canada (~1.15). The streamwater ratio values were ~1.16 to 1.17, while those for rainwater were as low as 1.09; in good agreement with the lower lake water values. This means that there must be an additional atmospheric source of Pb, which has a lower $^{206}Pb/^{207}Pb$ ratio than leaded on-road gasoline. Supporting evidence came from contemporary samples such as near surface peat, rainwater and snow, all of which confirmed a trend away from natural Pb (1.191 to 1.201) to lower $^{206}Pb/^{207}Pb$ ratios. The local smelting activities (Sudbury) were unlikely to be the source of anthropogenic Pb as Sudbury-derived emissions exhibit a typical $^{206}Pb/^{207}Pb$ ratio of ~1.15, similar to leaded on-road gasoline. Instead, it was suggested that long-range transport of Pb from the smelter at Rouyn-Noranda (known as the "Capital of Metal," NW Quebec) may still be impacting on Kawagama Lake but no Pb isotope data was quoted to support this supposition. Several studies, summarized in Mager (2012), reported Pb concentrations in matched reference and mining-disturbed streams in Missouri and the western U.S. They are summarized in Table 2-11.



Note: DENA = Denali (Alaska), MORA = Mount Rainier (Washington State), GLAC = Glacier (Montana), OLYM = Olympic (Washington State), NOAT = Noatak (Alaska).

Source: WACAP Database (NPS, 2011)

Figure 2-33 Boxplots of Pb concentration in surface waters measured at five National Parks and Preserves.

The range of Pb levels in various saltwater environments are available from several studies although the values are not specific to the U.S. A range of 0.005-0.4 µg Pb/L for seawater was reported by Leland and Kuwabara (1985) to reflect localized anthropogenic inputs in marine environments based on references from prior to 1980. A range of 0.01 to 27 µg Pb/L was reported for non-U.S. marine and estuarine waters by a group of publications dating from 1977 through 1990 that were summarized by Sadiq (1992). Boyle et al. (2005) compared measurements of Pb in near-surface ocean waters from the central north Pacific Ocean (north of Hawaii) from 1976 (Schaule and Patterson, 1981) with those from 1999 and observed a roughly 2-fold decrease in Pb concentration within the mixed layer (first 200 meters) from 13 ng/kg down to 7 ng/kg but no difference in concentrations deeper than 200 meters. In combination with measurements from Schaule and Patterson (1983) and Veron et al. (1993), Wu and Boyle (1997) observed a consistently decreasing trend in Pb concentration in the north Atlantic Ocean north of Bermuda, from 32 ng/kg in 1979 to 10 ng/kg in 1997. In general, Pb in seawater is higher

in coastal areas and estuaries since these locations are closer to sources of Pb contamination and loading from terrestrial systems (U.S. EPA, 2008b).

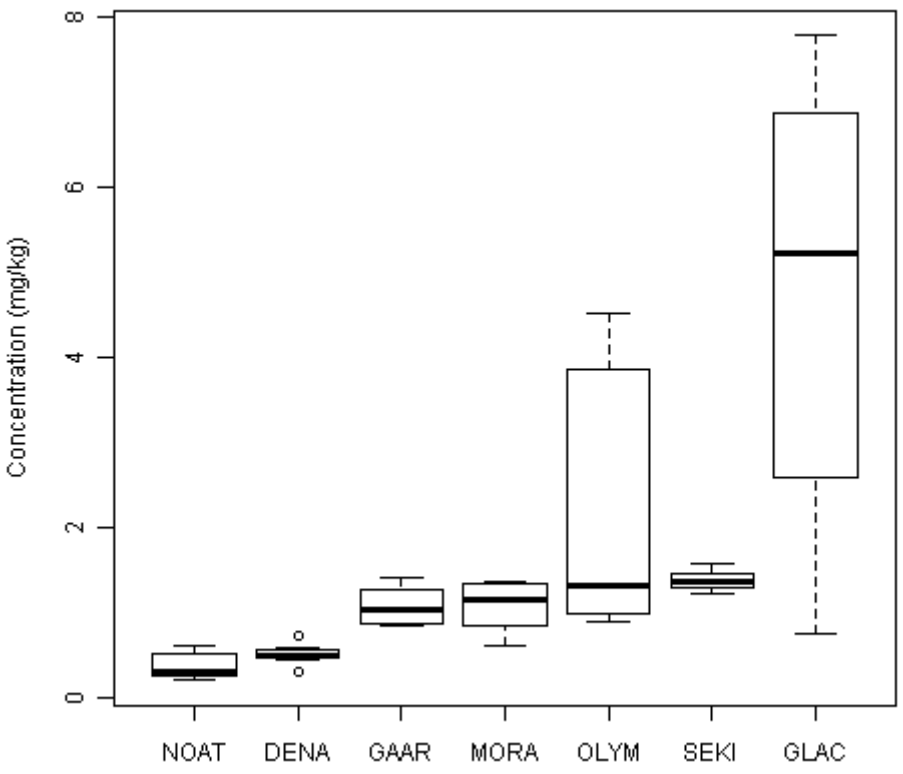
Table 2-11 Pb concentrations from stream food-webs; in mining-disturbed areas of Missouri and the western U.S.

Area	Total Pb in water (µg/L)	Dissolved Pb (µg/L)
Animas River, CO (Besser et al., 2001):		
Reference Streams	<1.8	<0.2
Mining-disturbed areas	0.9–8.6	<0.1–6.9
Boulder River, MT (Farag et al., 2007):		
Reference Streams	0.4 (colloidal)	0.3–0.4
Mining-disturbed areas	0.1–44	0.1–2
Coeur d'Alene River, ID (Clark, 2003; Farag et al., 1998):		
Reference Streams	2–20	0.01–2
Mining-disturbed areas	6–2,000	2–50
New Lead Belt, MO (Besser et al., 2007; Brumbaugh et al., 2007):		
Reference Streams	NR	<0.01-1.6
Mining-disturbed areas	NR	0.02–1.7

Adapted with permission of Elsevier: [Table 4.4 in Mager (2012)]

2.6.6 Vegetation

The 2006 Pb AQCD (U.S. EPA, 2006b) presented data on Pb in vegetation. The main conclusions were that Pb uptake was strongly affected by pH, and acidic soils are most likely to have Pb in solution for absorption by plants. Additionally, the 2006 Pb AQCD (U.S. EPA, 2006b) states that most Pb stored within vegetation is stored in roots rather than fruits or shoots. Recent measurements from the WACAP study (NPS, 2011) have shown some Pb storage in lichens. Median Pb concentrations ranged from 0.3 mg/kg in Noatak National Park (Alaska) to 5 mg/kg in Glacier National Park (Montana), with substantial variation in the Glacier and Olympic National Park (Washington State) samples (Figure 2-34). Landers et al. (2008) state that lichen Pb concentrations have decreased substantially from the 1980s and that metal concentrations were within background levels for these remote Western sites.



Note: DENA = Denali (Alaska), GAAR = Gates of the Arctic (Alaska), GLAC = Glacier (Montana), MORA = Mount Rainier (Washington State), NOAT = Noatak (Alaska), OLYM = Olympic (Washington State), SEKI = Sequoia and Kings Canyon (California).

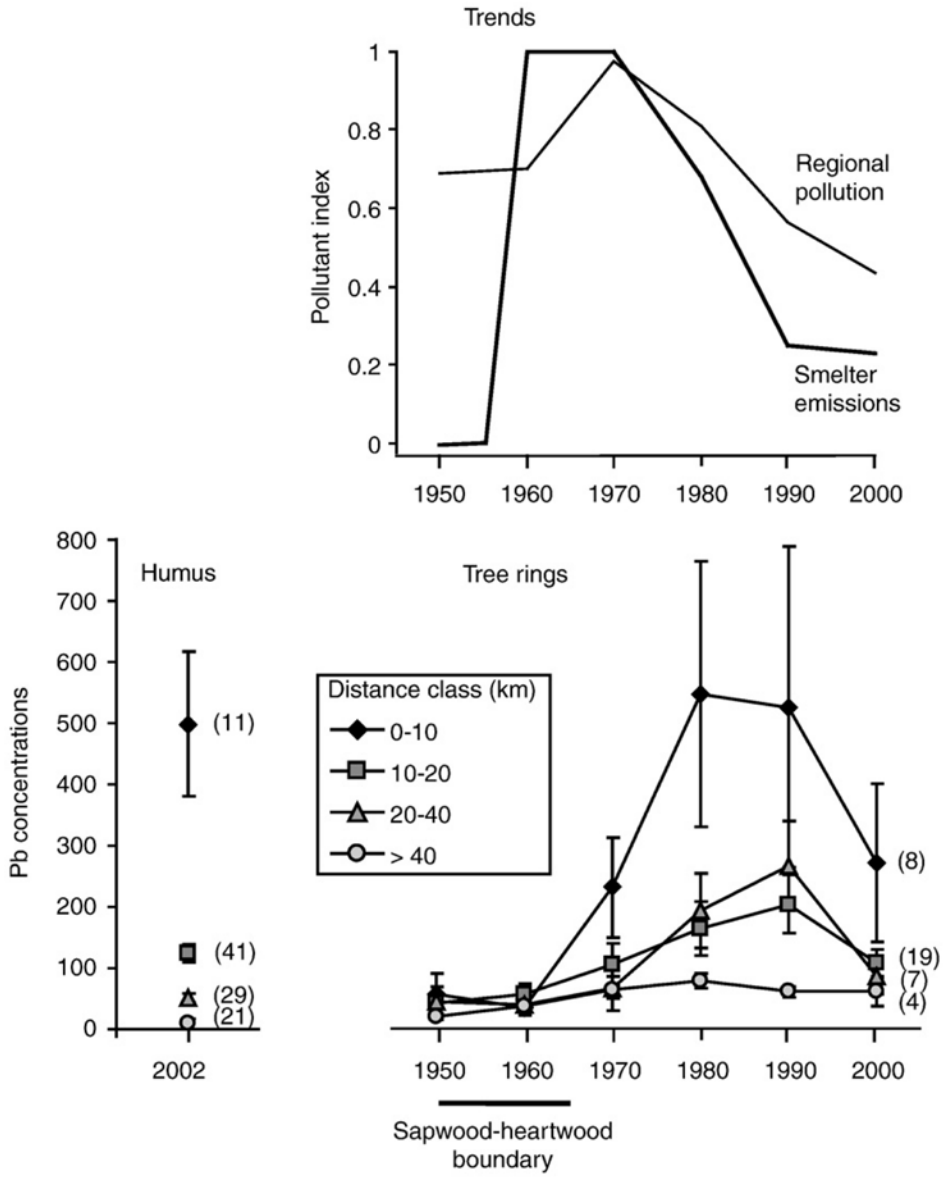
Source: WACAP Database (NPS, 2011)

Figure 2-34 Boxplots of Pb concentration in lichen measured at seven National Parks and Preserves.

Mosses can be used effectively for monitoring trends in Pb deposition as demonstrated in many studies (Harmens et al., 2010; Harmens et al., 2008). For example, Harmens et al. (2008) showed that a 52% decrease in deposited Pb concentrations corresponded to a 57% decrease in Pb concentrations in moss. Farmer et al. (2010) showed that there was good agreement between the $^{206}Pb/^{207}Pb$ ratio for precipitation and mosses collected in northeast Scotland. A study in the Vosges Mountains (France) also found a ratio value of 1.158 for a moss sample and a surface soil litter value of 1.167 and concluded that 1.158 to 1.167 represented the current atmospheric baseline (Geagea et al., 2008). For rural northeast Scotland, a combination of sources is giving rise to a $^{206}Pb/^{207}Pb$ ratio of ~1.15 in recent precipitation and mosses (Farmer et al., 2010). Clearly, sources with a lower ratio than coal (~1.20) must be contributing substantially to the overall emissions. Pb from waste incineration has been implicated as a possible current source (cf. typical $^{206}Pb/^{207}Pb$ ratios for Pb from European incineration plants are ~1.14 to 1.15 [de la Cruz et al. (2009) and references therein].

Pb has been measured on vegetation near roads in recent years. Hasselbach et al. (2005) measured Pb and other metals in mosses to assess deposition of metals along a haul road leading from a port to the Red Dog Zn-Pb mine in Northwest Alaska. They observed that moss concentrations of Pb decreased with increasing distance from the road, while subsurface soils (average depth = 62 meters) did not vary with distance from the road. The strong moss Pb gradient and constant subsurface soil Pb concentrations imply that Pb concentrations in mosses were primarily attributed to deposition and did not have appreciable contributions from soil. Throughout the study area, median moss Pb concentration was 16.2 mg/kg (dry basis), with a range of 1.1-912.5 mg/kg. Concentrations along the port road also diminished with increasing distance from the port, where ore loading operations take place. Hasselbach et al. (2005) attributed the concentrations to ore dust generated during loading operations at the port and mine along with fugitive dust escaping during truck transport. Maher et al. (2008) measured average Pb loading onto tree leaves near highways to be 29 µg/m^2 (max: 81 µg/m^2) at elevations ranging from 0.30 to 2.1 meters.

Trends in Pb concentration among flora have decreased in recent years. For example, Franzaring et al. (2010) evaluated data from a 20-year biological monitoring study of Pb concentration in permanent forest and grassland plots in Baden-Württemberg, southwest Germany. Grassland and tree foliage samples were collected from 1985-2006. The samples were not washed and so atmospheric deposition rather than uptake from the soil probably predominates. For all foliage (beech and spruce), Pb concentrations have shown large reductions over time, particularly in the early 1990s. The Pb concentrations in the grassland vegetation also decreased from the late 1980s to the early 1990s but the trend thereafter was found to be statistically non-significant. The reduction corresponded to the phase-out of leaded on-road gasoline in Germany. Similarly, Aznar et al. (2008a) observed that the decline in Pb concentrations in the outer level of tree rings corresponded with the decline in Cu smelter emissions in Gaspé Peninsula in Canada; Figure 2-35. Both Pb concentrations and Pb isotope ratios declined with distance from the smelter (Aznar et al., 2008b; Aznar et al., 2008a).



Notes: Humus Pb concentration reported in units of mg/kg dry basis, and tree ring Pb concentration reported in units of µg/kg dry basis.

Source: Reprinted with permission of Elsevier Publishing, Aznar et al. (2008a)

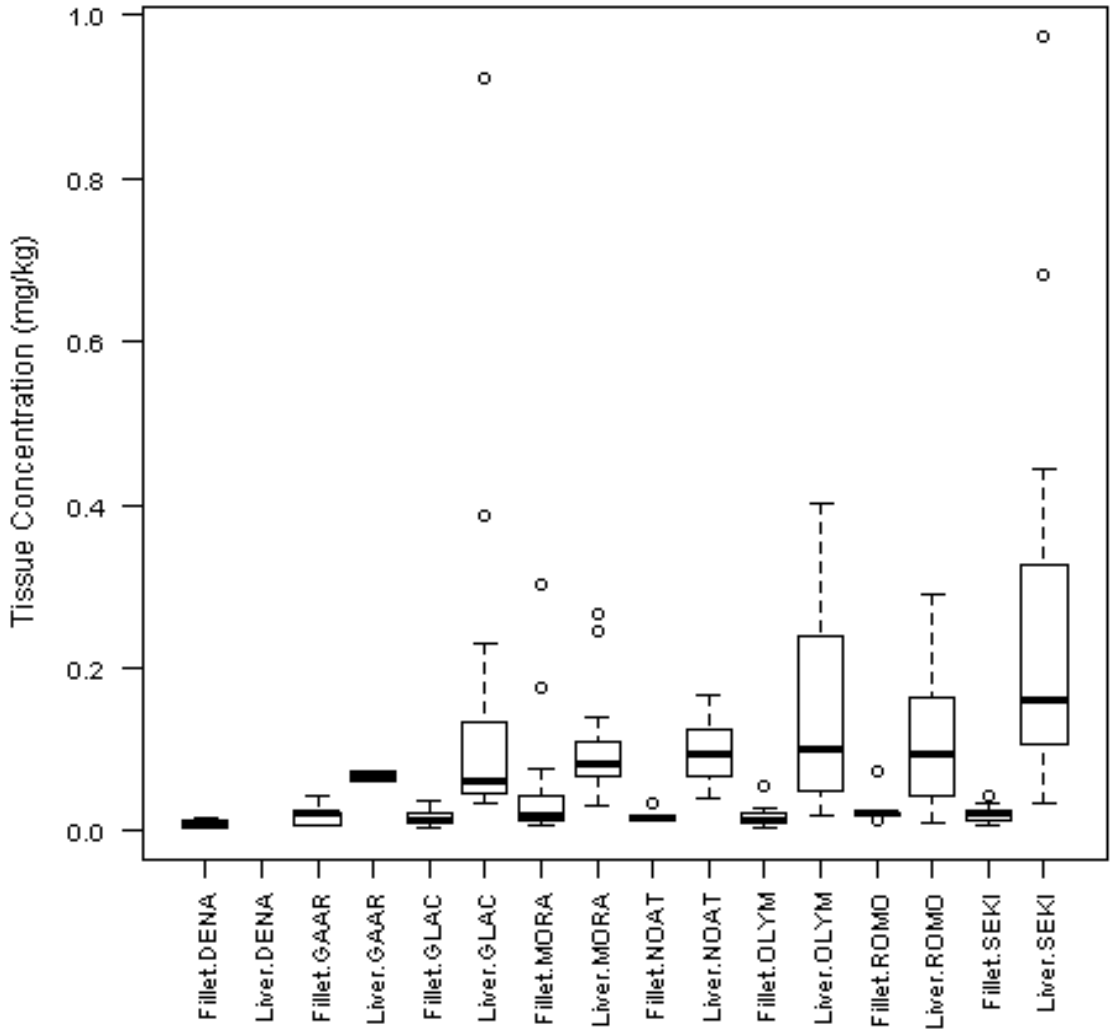
Figure 2-35 Trends in regional pollution near a copper (Cu) smelter in Canada and Pb concentrations at the boundary of heartwood trees within roughly 75 km of the smelter.

2.6.7 Aquatic Bivalves

Data from invertebrate waterborne populations can serve as in indicator of Pb contamination because animals such as mussels and oysters take in contaminants during filter feeding. Kimbrough et al. (2008) surveyed Pb concentrations in mussels, zebra mussels, and oysters along the coastlines of the continental U.S. In general, they observed the highest concentrations of Pb in the vicinity of urban and industrial areas. Company et al. (2008) measured Pb concentrations and Pb isotope ratios in bivalves along the Guadiana River separating Spain and Portugal. Analysis of Pb isotope ratio data suggested that high Pb concentrations were related to historical mining activities in the region. Elevated Pb concentrations were also observed by Company et al. (2008) in the vicinity of more populated areas. Couture et al. (2010) report data from a survey of the isotopic ratios of Pb in *Mytilus edulis* blue mussel, collected off the coast of France from 1985-2005. The results indicated that the likely source of Pb in mussel tissue is from resuspension of contaminated sediments enriched with Pb runoff from wastewater treatment plants, municipal waste incinerators, smelters and refineries rather than from atmospheric deposition (Couture et al., 2010).

2.6.8 Vertebrate Populations

Pb concentrations in fish fillet and liver were measured through the WACAP study in eight National Parks and Preserves (NPS, 2011). For fish fillet, Pb concentrations ranged from 0.0033-0.30 mg/kg dry basis, with a median of 0.016 mg/kg dry basis. Liver stores were several times higher, with Pb concentrations ranging from 0.011-0.97 mg/kg dry basis and a median of 0.096 mg/kg dry basis. Pb concentrations in moose meat and liver were also measured at the Denali National Park and Preserve (Alaska) as part of WACAP (NPS, 2011). Moose meat Pb concentrations ranged from 0.021-0.23 mg/kg dry basis with a median of 0.037 mg/kg dry basis. Pb concentrations in moose liver ranged from 0.025-0.11 mg/kg dry basis with a median of 0.053 mg/kg dry basis. Boxplots of measured Pb concentrations in fish fillet and liver are shown in Figure 2-36, and boxplots of measured Pb concentrations for moose meat and liver are shown in Figure 2-37. For fish and meat tissues, median and maximum Pb concentrations were substantially lower than values reported in the 2006 Pb AQCD (U.S. EPA, 2006b). Similarly, in a study of Pb levels in moose teeth from Isle Royale, MI, (Vucetich et al., 2009) median and mean Pb levels underwent a statistically significant decrease from the period 1952-1982 to 1983-2002 in both calves and adult moose. For 1952-1982, Pb concentrations were relatively constant, and a linear decline ($R^2 = 0.86$) was observed for 1983-2002. These findings suggest an overall decline but still some Pb accumulation in fish and moose in these remote locations occurring recently.

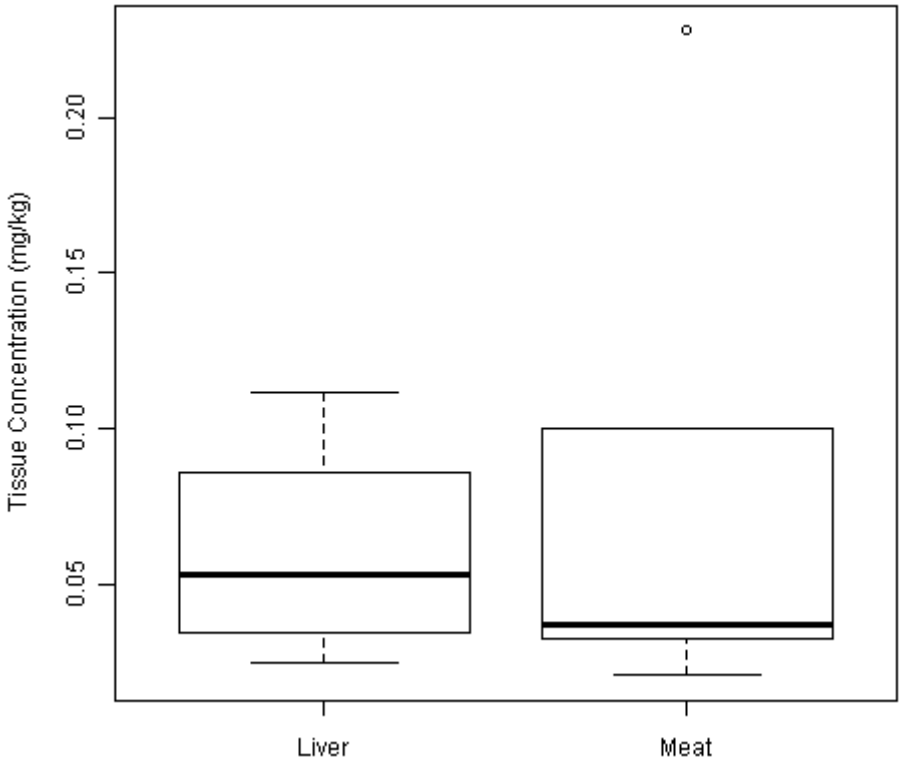


DENA = Denali (Alaska), GAAR = Gates of the Arctic (Alaska), GLAC = Glacier (Montana), MORA = Mount Rainier (Washington State), NOAT = Noatak (Alaska), OLYM = Olympic (Washington State), ROMO = Rocky Mountain (Colorado), SEKI = Sequoia and Kings Canyon (California).

Note: Tissue concentration reported on a dry basis.

Source: WACAP Database (NPS, 2011)

Figure 2-36 Boxplots of Pb concentration in fish fillet and fish liver, measured at eight National Parks and/or Preserves.



Note: Tissue concentration reported on a dry basis.
Source: WACAP Database (NPS, 2011)

Figure 2-37 Boxplots of Pb concentration in moose meat and moose liver measured at Denali National Park and Preserve (Alaska).

2.7 Summary and Conclusions

2.7.1 Sources of Atmospheric Pb

The 2006 Pb AQCD (U.S. EPA, 2006b) documented the decline in ambient air Pb emissions following the ban on alkyl-Pb additives for on-road gasoline. Pb emissions declined by 98% from 1970 to 1995 and then by an additional 77% from 1995 to 2008, at which time national Pb emissions were 950 tons/year. As was the case for the 2008 NAAQS review, piston-engine aircraft emissions currently comprise the largest share (58%) of total atmospheric Pb emissions nationally (U.S. EPA, 2011a). Other sources of ambient air Pb, in approximate order of importance with regard to national totals, include metal working and mining, fuel combustion, other industrial sources, roadway related sources, and historic Pb. Although piston-engine aircraft collectively comprise the largest emissions source, the highest emitting individual industrial sites produce more ambient air Pb emissions than individual airports.

2.7.2 Fate and Transport of Pb

The atmosphere is the main environmental transport pathway for Pb, and on a global scale atmospheric Pb is primarily associated with fine PM. Pb in fine PM is transported long distances and found in remote areas. Atmospheric Pb deposition peaked in the 1970s, followed by a decline. Both wet and dry deposition are important removal mechanisms for atmospheric Pb. Wet deposition is more important for fine Pb, and Pb associated with coarse PM is usually removed by dry deposition. Local deposition fluxes are much higher near industrial sources, and a substantial amount of emitted Pb is deposited near sources, leading to increased soil Pb concentrations. After initial deposition, Pb may potentially be resuspended and redeposited many times before reaching a site where further transport is unlikely, especially for dry deposition.

In water, Pb is transported as free ions, soluble chelates, or on surfaces of Fe-rich and organic-rich colloids. In most surface waters, atmospheric deposition is the largest source of Pb, but urban runoff and industrial discharge are also considerable. A substantial portion of Pb in runoff ultimately originates from atmospheric deposition, but substantial amounts of Pb from vehicle wear and building materials can also be transported by runoff waters without becoming airborne. Often the majority of Pb transport by runoff occurs at the beginning of a rainfall event. Pb is rapidly dispersed in water, and highest concentrations of Pb are observed near sources where Pb is deposited.

Transport in surface waters is largely controlled by exchange with sediments. The cycling of Pb between water and sediments is governed by chemical, biological, and mechanical processes, which are affected by many factors. Organic matter in sediments has a high capacity for accumulating trace elements like Pb. Binding of anoxic sediments to sulfides is a particularly important process that affects Pb bioavailability. Pb is relatively stable in sediments, with long residence times and limited mobility. However, Pb-containing sediment particles can be remobilized into the water column. Resuspended Pb is largely associated with OM or Fe and Mn particles. This resuspension of contaminated sediments strongly influences the lifetime of Pb in water bodies and can be a more important Pb source to the water column than atmospheric deposition. Resuspension of sediments largely occurs during discrete events related to storms.

A complex variety of factors influence Pb retention in soil, including hydraulic conductivity, solid composition, OM content, clay mineral content, microbial activity, plant root channels, animal holes, geochemical reactions, colloid amounts, colloidal surface charge, and pH. Leaf litter can be an important temporary sink for metals from the soil around and below leaves, and decomposition of leaf litter can reintroduce substantial amounts of Pb into soil "hot spots," where re-adsorption of Pb is favored. A small fraction of Pb in soil is present as the free Pb^{2+} ion. The fraction of Pb in this form is strongly dependent on soil pH.

In summary, environmental distribution of Pb occurs mainly through the atmosphere, from where it is deposited into surface waters and soil. Pb associated with coarse PM deposits to a great extent near sources, while fine Pb-PM can be transported long distances. Surface waters act as an important reservoir, with half-lives of Pb in the water column largely controlled by rates of deposition to and resuspension from bottom sediments. Pb retention in soil depends on Pb speciation and a variety of factors intrinsic to the soil.

2.7.3 Ambient Pb Monitoring

Since the publication of the 2006 Pb AQCD (U.S. EPA, 2006b) there has been little progress in the state of the science regarding monitoring technology and monitor siting criteria for representation of population exposures to airborne Pb and Pb of atmospheric origin. Our understanding of sampling errors in the existing FRM, of possible alternatives to existing Pb-TSP sampling technology, and of particle size ranges of Pb particles occurring in different types of locations have changed little in that time. In addition to monitors used historically for sampling Pb-PM, several single stage and multi-stage impactors and inlets used for sampling PM are also potential options for monitoring Pb

particles smaller than 15 μm. Ambient air Pb deposits onto soil or dust. As described in Section 3.1.1.1, the size distribution of dust and soil Pb particles is larger than the size distribution of ambient air Pb particles.

The current Pb monitoring network design requirements include two types of monitoring sites: source-oriented and non-source-oriented. Source-oriented monitoring sites are required near sources of air Pb emissions which are expected to or have been shown to contribute to ambient air Pb concentrations in excess of the NAAQS. Non-source-oriented monitoring of Pb-TSP or Pb-PM_{10} is also required at NCore sites in CBSAs with a population of at least 500,000.

In addition to Pb-TSP monitoring for the purposes of judging attainment with the NAAQS, Pb is also routinely measured in smaller PM fractions in the CSN, IMPROVE, and the NATTS networks. While monitoring in multiple networks provides extensive geographic coverage, measurements between networks are not directly comparable in all cases because different PM size ranges are sampled in different networks. Depending on monitoring network, Pb is monitored in TSP, PM_{10}, or $PM_{2.5}$ using high-volume or low-volume samplers.

2.7.4 Ambient Air Pb Concentrations

Ambient air Pb concentrations have declined drastically over the period 1980-2010. The median annual maximum 3-month average concentration of Pb-TSP has dropped by 97% from 0.87 $\mu g/m^3$ in 1980 to 0.03 $\mu g/m^3$ in 2010. The decline can be attributed to the phase-out of Pb antiknock agents in on-road fuel and reductions in industrial use and processing of Pb, as described in Section 2.2.1. The mean of maximum 3-month average concentrations for source-oriented monitors was skewed toward the 75th percentile of the data distribution and exceeded the level of the NAAQS, indicating that highest ambient air Pb concentrations occur near a subset of source-oriented monitors. Studies in the peer-reviewed literature have shown slightly elevated Pb concentrations downwind of industrial sources and airports.

Spatial variability was observed in ratios and correlations of Pb within different size fractions. Urban or suburban land types did not appear to affect sampled size distributions. Studies in the peer-reviewed literature suggest that proximity to industrial sources or some roadways can affect the Pb-PM size distribution. Pb concentrations exhibit varying degrees of association with other criteria pollutant concentrations. Overall, non-source Pb-TSP was moderately associated with CO, $PM_{2.5}$, and PM_{10}, which may indicate some role of traffic in Pb exposure. Among trace metals speciated from $PM_{2.5}$, Pb was not associated with most pollutants; Pb did associate with Zn,

although that association was low-to-moderate, suggesting mobile source emissions contributing to the Pb. EC, Cu, OC, Br, and K concentrations also exhibited low-to-moderate associations with Pb concentrations. Such correlations may suggest some common sources affecting the pollutants. Finally, the evidence on natural background Pb suggests a plausible background airborne Pb range of 0.02 to 1 ng/m^3.

2.7.5 Ambient Pb Concentrations in Non-Air Media and Biota

Atmospheric deposition has led to measurable Pb concentrations observed in rain, snowpack, soil, surface waters, sediments, agricultural plants, livestock, and wildlife across the world, with highest concentrations near Pb sources, such as metal smelters. Since the phase-out of Pb from on-road gasoline, concentrations in these media have decreased to varying degrees. In rain, snowpack, and surface waters, Pb concentrations have decreased considerably. Declining Pb concentrations in tree foliage, trunk sections, and grasses have also been observed. In contrast, Pb is retained in soils and sediments, where it provides a historical record of deposition and associated ambient concentrations. In remote lakes, sediment profiles indicate higher Pb concentrations in near surface sediment as compared to pre-industrial era sediment from greater depth and indicate peak concentrations between 1960 and 1980, when leaded on-road gasoline was at peak use. Concentrations of Pb in moss, lichens, peat, and aquatic bivalves have been used to understand spatial and temporal distribution patterns of air Pb concentrations. Ingestion and water intake are the major routes of Pb exposure for aquatic organisms, and food, drinking water, and inhalation are major routes of exposure for livestock and terrestrial wildlife. Overall, Pb concentrations have decreased substantially in media through which Pb is rapidly transported, such as air and water. Substantial Pb remains in soil and sediment sinks. In areas less affected by major local sources, the highest concentrations are below the surface layers and reflect the previous use of Pb in on-road gasoline and emissions reductions from other sources.

2.8 Chapter 2 Appendix (Supplemental Material)

2.8.1 Variability across the U.S.

Table 2-12 Distribution of 1-month average Pb-TSP concentrations (µg/m^3) nationwide, source-oriented monitors, 2008-2010.

Year	Season	State/ County	State	County name	Site ID	N: mo means	N sites	Mean	Min	1	5	10	25	50	75	90	95	99	max
Nationwide statistics																			
2008-2010						2,318		0.202	0.000	0.003	0.006	0.010	0.029	0.063	0.217	0.578	0.856	1.576	4.440
2008						548		0.318	0.004	0.004	0.013	0.024	0.050	0.110	0.348	0.841	1.240	2.557	4.440
2009						629		0.212	0.002	0.004	0.008	0.013	0.038	0.084	0.256	0.611	0.856	1.357	2.438
2010						1141		0.141	0.000	0.002	0.005	0.008	0.018	0.045	0.136	0.408	0.625	1.233	1.828
	Winter					554		0.202	0.000	0.002	0.006	0.008	0.026	0.055	0.184	0.502	0.883	2.438	3.103
	Spring					579		0.239	0.000	0.003	0.007	0.012	0.034	0.070	0.272	0.738	0.977	1.905	3.123
	Summer					601		0.186	0.001	0.003	0.006	0.010	0.030	0.066	0.212	0.559	0.755	1.233	4.440
	Fall					584		0.184	0.000	0.004	0.007	0.011	0.026	0.064	0.206	0.505	0.758	1.178	4.225
Nationwide statistics, pooled by site																			
2008-2010							111	0.161	0.002	0.003	0.008	0.013	0.031	0.056	0.177	0.441	0.687	0.997	1.275
2008							47	0.323	0.007	0.007	0.022	0.028	0.055	0.148	0.419	0.890	1.205	1.540	1.540
2009							54	0.214	0.007	0.007	0.013	0.018	0.043	0.090	0.343	0.669	0.849	0.921	0.921
2010							101	0.140	0.002	0.003	0.005	0.013	0.030	0.052	0.165	0.392	0.586	0.888	1.185
	Winter						108	0.156	0.000	0.003	0.006	0.009	0.021	0.048	0.160	0.475	0.879	1.130	1.488
	Spring						110	0.185	0.002	0.002	0.010	0.015	0.027	0.057	0.210	0.568	0.921	1.189	1.548

Table 2-12 (Continued): Distribution of 1-month average Pb-TSP concentrations (µg/m^3) nationwide, source-oriented monitors, 2008-2010.

Year	Season	State/ County	State	County name	Site ID	N: mo means	N sites	Mean	Min	1	5	10	25	50	75	90	95	99	max
	Summer						111	0.148	0.002	0.003	0.006	0.012	0.025	0.050	0.153	0.430	0.696	0.882	1.031
	Fall						110	0.152	0.002	0.004	0.009	0.013	0.034	0.062	0.168	0.421	0.616	1.081	1.189
Statistics for individual counties (2008-2010)																			
		01109	AL	Pike		32	1	0.5252	0.054	0.054	0.083	0.164	0.252	0.402	0.798	1.053	1.117	1.277	1.277
		06037	CA	Los Angeles		131	4	0.2380	0.018	0.019	0.026	0.034	0.047	0.085	0.246	0.602	0.905	2.501	2.880
		12057	FL	Hillsborough		81	3	0.1755	0.007	0.007	0.017	0.020	0.053	0.104	0.187	0.530	0.567	1.007	1.007
		13015	GA	Bartow		12	1	0.0128	0.007	0.007	0.007	0.008	0.008	0.014	0.016	0.017	0.019	0.019	0.019
		13215	GA	Muscogee		12	1	0.0361	0.004	0.004	0.004	0.010	0.013	0.027	0.043	0.058	0.140	0.140	0.140
		17031	IL	Cook		11	1	0.1515	0.028	0.028	0.028	0.028	0.050	0.074	0.196	0.304	0.580	0.580	0.580
		17115	IL	Macon		12	1	0.0800	0.018	0.018	0.018	0.025	0.035	0.074	0.118	0.144	0.168	0.168	0.168
		17119	IL	Madison		36	1	0.1367	0.018	0.018	0.022	0.024	0.037	0.068	0.175	0.304	0.363	0.836	0.836
		17143	IL	Peoria		24	2	0.0119	0.010	0.010	0.010	0.010	0.010	0.010	0.012	0.016	0.023	0.024	0.024
		17195	IL	Whiteside		12	1	0.0194	0.010	0.010	0.010	0.012	0.012	0.015	0.024	0.036	0.040	0.040	0.040
		17201	IL	Winnebago		11	1	0.0339	0.010	0.010	0.010	0.014	0.020	0.024	0.032	0.050	0.118	0.118	0.118
		18035	IN	Delaware		59	2	0.2746	0.034	0.034	0.040	0.049	0.080	0.128	0.241	0.427	1.011	4.440	4.440
		18089	IN	Lake		57	3	0.0309	0.004	0.004	0.007	0.008	0.012	0.020	0.035	0.052	0.079	0.298	0.298
		18097	IN	Marion		70	2	0.0195	0.003	0.003	0.005	0.005	0.008	0.012	0.025	0.046	0.050	0.125	0.125
		18127	IN	Porter		12	1	0.0125	0.004	0.004	0.004	0.005	0.007	0.009	0.021	0.024	0.026	0.026	0.026
		19155	IA	Pottawattamie		12	1	0.1536	0.025	0.025	0.025	0.026	0.063	0.164	0.257	0.276	0.282	0.282	0.282
		20169	KS	Saline		11	1	0.2020	0.043	0.043	0.043	0.044	0.083	0.133	0.320	0.457	0.488	0.488	0.488
		21019	KY	Boyd		7	1	0.0042	0.002	0.002	0.002	0.002	0.004	0.004	0.004	0.007	0.007	0.007	0.007
		21151	KY	Madison		12	1	0.0255	0.004	0.004	0.004	0.008	0.013	0.017	0.022	0.032	0.121	0.121	0.121
		26067	MI	Ionia		12	1	0.1781	0.016	0.016	0.016	0.023	0.054	0.169	0.279	0.361	0.414	0.414	0.414
		27003	MN	Anoka		12	1	0.0157	0.003	0.003	0.003	0.005	0.007	0.011	0.021	0.022	0.054	0.054	0.054
		27037	MN	Dakota		36	1	0.1966	0.037	0.037	0.048	0.058	0.084	0.137	0.259	0.424	0.572	0.738	0.738
		27145	MN	Stearns		12	1	0.0028	0.000	0.000	0.000	0.000	0.000	0.003	0.005	0.006	0.008	0.008	0.008
		29093	MO	Iron		171	7	0.3388	0.007	0.008	0.014	0.018	0.033	0.093	0.518	0.850	1.110	2.557	4.225

Table 2-12 (Continued): Distribution of 1-month average Pb-TSP concentrations (µg/m^3) nationwide, source-oriented monitors, 2008-2010.

Year	Season	State/ County	State	County name	Site ID	N: mo means	N sites	Mean	Min	1	5	10	25	50	75	90	95	99	max
		29099	MO	Jefferson		453	19	0.4795	0.011	0.015	0.033	0.048	0.141	0.336	0.659	1.118	1.451	2.220	3.123
		29179	MO	Reynolds		48	4	0.0428	0.007	0.007	0.008	0.011	0.017	0.027	0.060	0.087	0.099	0.268	0.268
		31053	NE	Dodge		9	1	0.0515	0.005	0.005	0.005	0.005	0.021	0.031	0.053	0.149	0.149	0.149	0.149
		31127	NE	Nemaha		8	1	0.0476	0.008	0.008	0.008	0.008	0.010	0.024	0.049	0.206	0.206	0.206	0.206
		36071	NY	Orange		105	3	0.0281	0.001	0.001	0.003	0.004	0.006	0.018	0.044	0.063	0.081	0.101	0.134
		39035	OH	Cuyahoga		72	3	0.0941	0.004	0.004	0.007	0.008	0.014	0.038	0.121	0.210	0.400	0.719	0.719
		39051	OH	Fulton		34	1	0.1462	0.009	0.009	0.009	0.026	0.057	0.091	0.170	0.420	0.490	0.510	0.510
		39091	OH	Logan		102	4	0.0480	0.003	0.003	0.004	0.005	0.020	0.042	0.070	0.090	0.100	0.120	0.170
		39101	OH	Marion		10	1	0.0358	0.025	0.025	0.025	0.026	0.027	0.033	0.041	0.054	0.066	0.066	0.066
		39151	OH	Stark		11	1	0.0175	0.008	0.008	0.008	0.009	0.010	0.018	0.024	0.025	0.028	0.028	0.028
		39155	OH	Trumbull		8	1	0.0075	0.004	0.004	0.004	0.004	0.005	0.007	0.008	0.017	0.017	0.017	0.017
		40121	OK	Pittsburg		11	1	0.0023	0.002	0.002	0.002	0.002	0.002	0.002	0.003	0.003	0.003	0.003	0.003
		41071	OR	Yamhill		12	1	0.0157	0.006	0.006	0.006	0.007	0.008	0.016	0.020	0.025	0.037	0.037	0.037
		42003	PA	Allegheny		24	2	0.0369	0.006	0.006	0.006	0.006	0.010	0.017	0.040	0.121	0.144	0.149	0.149
		42007	PA	Beaver		54	3	0.1130	0.042	0.042	0.044	0.047	0.068	0.096	0.128	0.198	0.272	0.286	0.286
		42011	PA	Berks		117	6	0.0989	0.034	0.035	0.038	0.042	0.048	0.066	0.119	0.200	0.295	0.347	0.348
		42045	PA	Delaware		12	1	0.0452	0.043	0.043	0.043	0.043	0.043	0.045	0.047	0.048	0.048	0.048	0.048
		42055	PA	Franklin		11	1	0.0449	0.042	0.042	0.042	0.043	0.043	0.045	0.047	0.047	0.047	0.047	0.047
		42063	PA	Indiana		12	1	0.0454	0.042	0.042	0.042	0.043	0.043	0.044	0.046	0.047	0.058	0.058	0.058
		42073	PA	Lawrence		8	1	0.0438	0.042	0.042	0.042	0.042	0.043	0.044	0.045	0.046	0.046	0.046	0.046
		42079	PA	Luzerne		10	1	0.0953	0.043	0.043	0.043	0.044	0.045	0.071	0.102	0.215	0.268	0.268	0.268
		42129	PA	Westmore-land		12	1	0.0439	0.041	0.041	0.041	0.041	0.043	0.044	0.045	0.046	0.047	0.047	0.047
		47093	TN	Knox		48	2	0.0165	0.002	0.002	0.005	0.006	0.008	0.012	0.019	0.032	0.038	0.063	0.063
		47163	TN	Sullivan		120	4	0.0534	0.021	0.023	0.030	0.032	0.037	0.045	0.059	0.083	0.124	0.145	0.156
		48085	TX	Collin		108	3	0.3062	0.007	0.028	0.040	0.052	0.104	0.189	0.438	0.717	0.904	1.178	1.564
		48375	TX	Potter		6	1	0.0044	0.004	0.004	0.004	0.004	0.004	0.004	0.005	0.006	0.006	0.006	0.006
		51770	VA	Roanoke City		12	1	0.0412	0.005	0.005	0.005	0.008	0.010	0.015	0.035	0.054	0.272	0.272	0.272

Table 2-12 (Continued): Distribution of 1-month average Pb-TSP concentrations (µg/m^3) nationwide, source-oriented monitors, 2008-2010.

Year	Season	State/ County	State	County name	Site ID	N: mo means	N sites	Mean	Min	1	5	10	25	50	75	90	95	99	max
		55117	WI	Sheboygan		12	1	0.0802	0.001	0.001	0.001	0.003	0.007	0.054	0.136	0.182	0.279	0.279	0.279
		72013	PR	Arecibo (Puerto Rico)		12	1	0.1774	0.038	0.038	0.038	0.064	0.102	0.178	0.264	0.290	0.310	0.310	0.310
Statistics for individual sites where overall average monthly mean ≥ national 90th percentile (2008-2010)																			
					11090003	32		0.525	0.054	0.054	0.083	0.164	0.252	0.402	0.798	1.053	1.117	1.277	1.277
					060371405	36		0.671	0.100	0.100	0.188	0.235	0.285	0.359	0.771	2.086	2.501	2.880	2.880
					290930016	36		0.670	0.166	0.166	0.186	0.219	0.330	0.466	0.726	0.974	2.435	4.225	4.225
					290930021	36		0.681	0.082	0.082	0.084	0.095	0.194	0.650	0.879	1.437	2.438	2.557	2.557
					290990004	36		0.997	0.256	0.256	0.307	0.408	0.598	0.918	1.236	1.690	1.905	2.416	2.416
					290990015	21		1.275	0.340	0.340	0.421	0.646	0.756	1.118	1.349	2.440	3.103	3.123	3.123
					290990020[a]	31		0.687	0.191	0.191	0.195	0.297	0.368	0.620	0.808	1.111	1.280	2.220	2.220
					290990021[a]	21		0.719	0.084	0.084	0.141	0.359	0.572	0.666	0.876	1.164	1.168	1.553	1.553
					290990022[a]	31		0.441	0.140	0.140	0.171	0.208	0.303	0.409	0.599	0.683	0.754	0.861	0.861
					290999001[a]	24		0.850	0.186	0.186	0.208	0.319	0.449	0.845	1.071	1.382	1.558	1.623	1.623
					290999005[a]	24		0.986	0.155	0.155	0.250	0.330	0.558	0.864	1.487	1.802	1.828	1.985	1.985
					480850009[a]	36		0.601	0.137	0.137	0.138	0.185	0.420	0.579	0.757	1.101	1.178	1.564	1.564

[a]Sites listed in the bottom six rows of this table fall in the upper 90th percentile of the data pooled by site.

Table 2-13 Distribution of 1-month average Pb-TSP concentrations (µg/m^3) nationwide, non-source-oriented monitors, 2008-2010.

Year	Season	State/ County	State	County name	Site ID	N: mo means	N sites	Mean	Min	1	5	10	25	50	75	90	95	99	max
Nationwide statistics																			
2008-2010						2290		0.0120	0.000	0.000	0.000	0.002	0.004	0.010	0.015	0.026	0.040	0.052	0.136
2008						685		0.0126	0.000	0.000	0.000	0.002	0.005	0.010	0.015	0.029	0.040	0.052	0.066
2009						768		0.0114	0.000	0.000	0.000	0.002	0.004	0.010	0.014	0.023	0.040	0.048	0.128
2010						837		0.0120	0.000	0.000	0.000	0.000	0.004	0.009	0.016	0.026	0.036	0.054	0.136
	Winter					556		0.0109	0.000	0.000	0.000	0.001	0.004	0.008	0.013	0.022	0.038	0.056	0.087
	Spring					574		0.0122	0.000	0.000	0.000	0.002	0.004	0.009	0.015	0.028	0.040	0.052	0.128
	Summer					584		0.0119	0.000	0.000	0.000	0.002	0.005	0.010	0.016	0.026	0.040	0.050	0.057
	Fall					576		0.0129	0.000	0.000	0.000	0.002	0.005	0.010	0.016	0.026	0.040	0.053	0.136
Nationwide statistics, pooled by site																			
2008-2010							88	0.0120	0.000	0.000	0.001	0.002	0.005	0.011	0.016	0.024	0.033	0.046	0.046
2008							59	0.0125	0.001	0.001	0.002	0.003	0.006	0.010	0.016	0.024	0.043	0.051	0.051
2009							66	0.0116	0.000	0.000	0.001	0.002	0.004	0.010	0.014	0.024	0.032	0.050	0.050
2010							73	0.0119	0.000	0.000	0.001	0.001	0.005	0.010	0.018	0.023	0.028	0.046	0.046
	Winter						88	0.0115	0.000	0.000	0.001	0.001	0.004	0.009	0.016	0.025	0.038	0.048	0.048
	Spring						86	0.0119	0.000	0.000	0.001	0.002	0.004	0.009	0.016	0.027	0.032	0.059	0.059
	Summer						88	0.0117	0.000	0.000	0.000	0.001	0.005	0.010	0.016	0.026	0.034	0.043	0.043
	Fall						88	0.0130	0.000	0.000	0.001	0.003	0.005	0.011	0.017	0.028	0.031	0.054	0.054
Statistics for individual counties (2008-2010)																			
		04013	AZ	Maricopa		6	1	0.0218	0.009	0.009	0.009	0.009	0.014	0.021	0.028	0.038	0.038	0.038	0.038
		06025	CA	Imperial		33	1	0.0162	0.004	0.004	0.006	0.009	0.011	0.015	0.019	0.025	0.032	0.035	0.035
		06037	CA	Los Angeles		224	8	0.0098	0.000	0.000	0.000	0.002	0.006	0.010	0.012	0.017	0.020	0.038	0.044

Table 2-13 (Continued): Distribution of 1-month average Pb-TSP concentrations (µg/m^3) nationwide, non-source-oriented monitors, 2008-2010.

Year	Season	State/ County	State	County name	Site ID	N: mo means	N sites	Mean	Min	1	5	10	25	50	75	90	95	99	max
		06065	CA	Riverside		72	2	0.0077	0.000	0.000	0.003	0.004	0.006	0.008	0.010	0.010	0.012	0.014	0.014
		06071	CA	San Bernardino		71	2	0.0091	0.001	0.001	0.003	0.004	0.007	0.010	0.012	0.014	0.014	0.022	0.022
		08005	CO	Arapahoe		9	1	0.0120	0.004	0.004	0.004	0.004	0.007	0.012	0.016	0.018	0.018	0.018	0.018
		08031	CO	Denver		12	1	0.0056	0.003	0.003	0.003	0.004	0.005	0.005	0.006	0.008	0.008	0.008	0.008
		13089	GA	DeKalb		10	1	0.0033	0.002	0.002	0.002	0.002	0.003	0.003	0.004	0.005	0.006	0.006	0.006
		17031	IL	Cook		288	8	0.0195	0.010	0.010	0.010	0.010	0.012	0.016	0.025	0.034	0.040	0.060	0.070
		17117	IL	Macoupin		24	1	0.0101	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.012	0.012
		17119	IL	Madison		36	1	0.0188	0.010	0.010	0.010	0.010	0.012	0.016	0.020	0.032	0.053	0.066	0.066
		17143	IL	Peoria		36	1	0.0105	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.013	0.013	0.014	0.014
		17163	IL	Saint Clair		36	1	0.0206	0.010	0.010	0.010	0.012	0.014	0.018	0.026	0.032	0.038	0.054	0.054
		18089	IN	Lake		36	1	0.0150	0.005	0.005	0.005	0.005	0.008	0.014	0.019	0.030	0.033	0.049	0.049
		18097	IN	Marion		35	1	0.0058	0.002	0.002	0.002	0.003	0.004	0.005	0.008	0.010	0.012	0.013	0.013
		18163	IN	Vanderburgh		33	2	0.0045	0.001	0.001	0.001	0.002	0.003	0.004	0.005	0.006	0.010	0.010	0.010
		25025	MA	Suffolk		31	2	0.0087	0.004	0.004	0.004	0.005	0.007	0.008	0.010	0.013	0.016	0.020	0.020
		26081	MI	Kent		12	1	0.0053	0.003	0.003	0.003	0.003	0.005	0.005	0.006	0.008	0.008	0.008	0.008
		26163	MI	Wayne		36	2	0.0112	0.003	0.003	0.003	0.004	0.005	0.009	0.015	0.021	0.023	0.032	0.032
		27017	MN	Carlton		12	1	0.0000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		27037	MN	Dakota		118	5	0.0035	0.000	0.000	0.000	0.000	0.000	0.002	0.005	0.008	0.010	0.017	0.036
		27053	MN	Hennepin		126	4	0.0032	0.000	0.000	0.000	0.000	0.000	0.002	0.005	0.006	0.008	0.010	0.044
		27075	MN	Lake		10	1	0.0000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
		27123	MN	Ramsey		71	3	0.0062	0.000	0.000	0.000	0.000	0.002	0.004	0.008	0.013	0.020	0.028	0.028
		27137	MN	Saint Louis		72	2	0.0015	0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.004	0.006	0.010	0.010
		27163	MN	Washington		72	3	0.0016	0.000	0.000	0.000	0.000	0.000	0.000	0.003	0.004	0.005	0.006	0.006
		29097	MO	Jasper		12	1	0.0125	0.007	0.007	0.007	0.007	0.009	0.012	0.017	0.018	0.019	0.019	0.019
		29187	MO	Saint Francois		24	2	0.0327	0.008	0.008	0.009	0.009	0.018	0.032	0.039	0.054	0.080	0.089	0.089
		29189	MO	Saint Louis		33	1	0.0230	0.005	0.005	0.005	0.005	0.006	0.008	0.050	0.050	0.050	0.066	0.066

Table 2-13 (Continued): Distribution of 1-month average Pb-TSP concentrations (µg/m^3) nationwide, non-source-oriented monitors, 2008-2010.

Year	Season	State/ County	State	County name	Site ID	N: mo means	N sites	Mean	Min	1	5	10	25	50	75	90	95	99	max
		36047	NY	Kings		24	1	0.0131	0.010	0.010	0.010	0.010	0.011	0.012	0.014	0.018	0.020	0.020	0.020
		39017	OH	Butler		34	1	0.0055	0.002	0.002	0.003	0.004	0.004	0.005	0.007	0.008	0.009	0.009	0.009
		39029	OH	Columbiana		107	3	0.0155	0.004	0.004	0.006	0.007	0.008	0.011	0.018	0.027	0.034	0.065	0.136
		39035	OH	Cuyahoga		107	3	0.0143	0.004	0.004	0.006	0.007	0.009	0.012	0.017	0.024	0.030	0.041	0.041
		39049	OH	Franklin		36	1	0.0092	0.004	0.004	0.005	0.005	0.007	0.009	0.011	0.013	0.014	0.016	0.016
		39143	OH	Sandusky		12	1	0.0048	0.003	0.003	0.003	0.003	0.004	0.005	0.006	0.006	0.007	0.007	0.007
		39167	OH	Washington		54	2	0.0048	0.002	0.002	0.002	0.003	0.003	0.005	0.006	0.007	0.008	0.010	0.010
		40115	OK	Ottawa		16	2	0.0124	0.003	0.003	0.003	0.005	0.006	0.013	0.017	0.021	0.025	0.025	0.025
		42003	PA	Allegheny		36	1	0.0105	0.000	0.000	0.000	0.000	0.004	0.009	0.015	0.019	0.024	0.053	0.053
		42021	PA	Cambria		23	1	0.0463	0.040	0.040	0.040	0.040	0.040	0.040	0.044	0.054	0.058	0.128	0.128
		42045	PA	Delaware		20	1	0.0432	0.040	0.040	0.040	0.040	0.040	0.043	0.046	0.047	0.048	0.048	0.048
		42101	PA	Philadelphia		24	1	0.0210	0.011	0.011	0.011	0.012	0.014	0.020	0.027	0.033	0.033	0.039	0.039
		42129	PA	Westmore-land		24	1	0.0419	0.037	0.037	0.040	0.040	0.040	0.040	0.042	0.050	0.050	0.053	0.053
		48061	TX	Cameron		35	1	0.0041	0.002	0.002	0.003	0.003	0.003	0.004	0.005	0.006	0.007	0.009	0.009
		48141	TX	El Paso		68	3	0.0206	0.014	0.014	0.014	0.014	0.015	0.017	0.019	0.029	0.056	0.087	0.087
		48201	TX	Harris		32	1	0.0053	0.003	0.003	0.003	0.004	0.004	0.005	0.006	0.007	0.008	0.010	0.010
		48479	TX	Webb		29	1	0.0134	0.004	0.004	0.005	0.006	0.008	0.011	0.018	0.026	0.028	0.035	0.035
		49035	UT	Salt Lake		12	1	0.0173	0.003	0.003	0.003	0.006	0.009	0.011	0.024	0.040	0.043	0.043	0.043
		51087	VA	Henrico		7	1	0.0066	0.003	0.003	0.003	0.003	0.003	0.004	0.005	0.024	0.024	0.024	0.024
Statistics for individual sites where overall average monthly mean ≥ national 90th percentile (2008-2010)																			
					170310022	36		0.0330	0.012	0.012	0.014	0.016	0.020	0.033	0.040	0.056	0.062	0.070	0.070
					170310026	36		0.0282	0.014	0.014	0.014	0.018	0.020	0.028	0.034	0.044	0.048	0.052	0.052
					170316003	36		0.0249	0.012	0.012	0.014	0.018	0.020	0.026	0.031	0.033	0.038	0.040	0.040
					291870006[a]	12		0.0383	0.009	0.009	0.009	0.015	0.024	0.035	0.042	0.080	0.089	0.089	0.089
					291870007[a]	12		0.0271	0.008	0.008	0.008	0.009	0.013	0.026	0.035	0.052	0.054	0.054	0.054
					420210808[a]	23		0.0463	0.040	0.040	0.040	0.040	0.040	0.040	0.044	0.054	0.058	0.128	0.128

Table 2-13 (Continued): Distribution of 1-month average Pb-TSP concentrations (µg/m^3) nationwide, non-source-oriented monitors, 2008-2010.

Year	Season	State/ County	State	County name	Site ID	N: mo means	N sites	Mean	Min	1	5	10	25	50	75	90	95	99	max
					420450002[a]	20		0.0432	0.040	0.040	0.040	0.040	0.040	0.043	0.046	0.047	0.048	0.048	0.048
					421290007[a]	24		0.0419	0.037	0.037	0.040	0.040	0.040	0.040	0.042	0.050	0.050	0.053	0.053
					481410002[a]	23		0.0236	0.016	0.016	0.016	0.016	0.017	0.018	0.021	0.033	0.056	0.087	0.087

[a]Sites listed in the bottom six rows of this table fall in the upper 90th percentile of the data pooled by site.

Table 2-14 Distribution of 3-month moving average Pb-TSP concentrations (µg/m^3) nationwide, source-oriented monitors, 2008-2010.

Year	Season	State/ County	State	County name	Site ID	N: mo means	N sites	Mean	Min	1	5	10	25	50	75	90	95	99	max
Nationwide statistics[a]																			
2008-2010						2,112		0.2134	0.000	0.004	0.010	0.014	0.035	0.079	0.250	0.600	0.881	1.555	2.889
2008						537		0.3225	0.005	0.006	0.016	0.028	0.056	0.129	0.385	0.900	1.197	2.452	2.889
2009						600		0.2177	0.004	0.005	0.011	0.016	0.040	0.090	0.292	0.622	0.799	1.217	2.070
2010						975		0.1507	0.000	0.002	0.008	0.012	0.024	0.052	0.173	0.436	0.694	1.055	1.375
	Winter					443		0.2366	0.003	0.004	0.011	0.014	0.040	0.083	0.272	0.647	0.963	2.070	2.621
	Spring					535		0.2376	0.000	0.004	0.011	0.014	0.035	0.078	0.323	0.642	0.999	2.017	2.889
	Summer					572		0.2022	0.002	0.003	0.009	0.015	0.034	0.077	0.240	0.580	0.869	1.261	2.163
	Fall					562		0.1835	0.002	0.004	0.009	0.013	0.033	0.078	0.220	0.521	0.714	1.186	2.456
Nationwide statistics, pooled by site																			
2008-2010							106	0.1671	0.002	0.003	0.012	0.015	0.030	0.059	0.173	0.577	0.717	1.009	1.316
2008							47	0.3309	0.007	0.007	0.024	0.029	0.056	0.154	0.461	0.814	1.284	1.639	1.639
2009							54	0.2203	0.007	0.007	0.013	0.019	0.042	0.086	0.311	0.632	0.840	0.886	0.886
2010							96	0.1415	0.002	0.002	0.008	0.013	0.027	0.053	0.163	0.407	0.619	1.110	1.110
	Winter						104	0.1700	0.003	0.004	0.011	0.013	0.025	0.055	0.171	0.522	0.827	1.097	1.324
	Spring						101	0.1904	0.001	0.002	0.013	0.016	0.028	0.060	0.186	0.502	0.874	1.231	1.740
	Summer						106	0.1597	0.002	0.004	0.010	0.015	0.028	0.058	0.174	0.520	0.788	0.989	1.104
	Fall						105	0.1538	0.002	0.004	0.009	0.012	0.032	0.066	0.170	0.462	0.630	0.960	1.161
Statistics for individual counties (2008-2010)																			
		01109	AL	Pike		25	1	0.5771	0.223	0.223	0.247	0.256	0.302	0.574	0.719	1.088	1.178	1.210	1.210
		06037	CA	Los Angeles		131	4	0.2521	0.023	0.023	0.036	0.041	0.055	0.078	0.237	0.543	0.832	2.452	2.489
		12057	FL	Hillsborough		79	3	0.1940	0.011	0.011	0.015	0.037	0.063	0.110	0.249	0.423	0.582	1.770	1.770
		13015	GA	Bartow		11	1	0.0125	0.009	0.009	0.009	0.009	0.011	0.013	0.014	0.015	0.016	0.016	0.016

Table 2-14 (Continued): Distribution of 3-month moving average Pb-TSP concentrations (µg/m^3) nationwide, source-oriented monitors, 2008-2010.

Year	Season	State/ County	State	County name	Site ID	N: mo means	N sites	Mean	Min	1	5	10	25	50	75	90	95	99	max
		13215	GA	Muscogee		12	1	0.0367	0.014	0.014	0.014	0.020	0.022	0.031	0.052	0.066	0.070	0.070	0.070
		17031	IL	Cook		9	1	0.1364	0.068	0.068	0.068	0.068	0.109	0.135	0.150	0.241	0.241	0.241	0.241
		17115	IL	Macon		10	1	0.0806	0.048	0.048	0.048	0.052	0.067	0.080	0.088	0.117	0.123	0.123	0.123
		17119	IL	Madison		36	1	0.1346	0.027	0.027	0.035	0.036	0.063	0.113	0.207	0.283	0.341	0.416	0.416
		17143	IL	Peoria		20	2	0.0121	0.010	0.010	0.010	0.010	0.011	0.012	0.014	0.015	0.016	0.016	0.016
		17195	IL	Whiteside		10	1	0.0191	0.012	0.012	0.012	0.014	0.016	0.019	0.022	0.025	0.025	0.025	0.025
		17201	IL	Winnebago		9	1	0.0356	0.019	0.019	0.019	0.019	0.021	0.027	0.057	0.063	0.063	0.063	0.063
		18035	IN	Delaware		57	2	0.2866	0.053	0.053	0.059	0.073	0.090	0.159	0.246	0.495	1.867	2.163	2.163
		18089	IN	Lake		46	2	0.0305	0.007	0.007	0.011	0.012	0.016	0.027	0.036	0.040	0.057	0.129	0.129
		18097	IN	Marion		66	2	0.0198	0.005	0.005	0.006	0.007	0.011	0.014	0.025	0.036	0.043	0.079	0.079
		18127	IN	Porter		10	1	0.0131	0.007	0.007	0.007	0.007	0.007	0.013	0.017	0.020	0.022	0.022	0.022
		19155	IA	Pottawattamie		12	1	0.1581	0.034	0.034	0.034	0.067	0.113	0.153	0.220	0.246	0.263	0.263	0.263
		20169	KS	Saline		9	1	0.2286	0.096	0.096	0.096	0.096	0.107	0.231	0.324	0.421	0.421	0.421	0.421
		21151	KY	Madison		10	1	0.0212	0.013	0.013	0.013	0.014	0.015	0.017	0.024	0.037	0.049	0.049	0.049
		26067	MI	Ionia		10	1	0.1980	0.106	0.106	0.106	0.110	0.128	0.212	0.259	0.273	0.284	0.284	0.284
		27003	MN	Anoka		10	1	0.0161	0.006	0.006	0.006	0.008	0.010	0.013	0.022	0.029	0.031	0.031	0.031
		27037	MN	Dakota		36	1	0.2026	0.068	0.068	0.072	0.088	0.104	0.216	0.248	0.357	0.415	0.429	0.429
		27145	MN	Stearns		10	1	0.0032	0.000	0.000	0.000	0.001	0.002	0.004	0.004	0.005	0.005	0.005	0.005
		29093	MO	Iron		158	6	0.3465	0.010	0.011	0.019	0.022	0.033	0.142	0.549	0.901	1.167	2.076	2.456
		29099	MO	Jefferson		423	19	0.4925	0.023	0.033	0.050	0.071	0.187	0.385	0.723	0.989	1.186	2.017	2.889
		29179	MO	Reynolds		40	4	0.0397	0.012	0.012	0.014	0.015	0.017	0.031	0.057	0.087	0.089	0.100	0.100
		31053	NE	Dodge		7	1	0.0474	0.019	0.019	0.019	0.019	0.020	0.060	0.067	0.072	0.072	0.072	0.072
		31127	NE	Nemaha		6	1	0.0447	0.019	0.019	0.019	0.019	0.024	0.032	0.075	0.087	0.087	0.087	0.087
		36071	NY	Orange		99	3	0.0271	0.003	0.003	0.004	0.005	0.007	0.027	0.037	0.068	0.075	0.086	0.086
		39035	OH	Cuyahoga		70	3	0.0905	0.006	0.006	0.010	0.011	0.021	0.050	0.122	0.221	0.287	0.531	0.531
		39051	OH	Fulton		30	1	0.1609	0.025	0.025	0.027	0.046	0.054	0.092	0.254	0.354	0.453	0.567	0.567
		39091	OH	Logan		100	4	0.0499	0.004	0.004	0.004	0.006	0.033	0.047	0.072	0.090	0.095	0.100	0.100

Table 2-14 (Continued): Distribution of 3-month moving average Pb-TSP concentrations (µg/m^3) nationwide, source-oriented monitors, 2008-2010.

Year	Season	State/ County	State	County name	Site ID	N: mo means	N sites	Mean	Min	1	5	10	25	50	75	90	95	99	max
		39101	OH	Marion		8	1	0.0379	0.032	0.032	0.032	0.032	0.034	0.037	0.042	0.047	0.047	0.047	0.047
		39151	OH	Stark		9	1	0.0180	0.015	0.015	0.015	0.015	0.016	0.018	0.019	0.023	0.023	0.023	0.023
		39155	OH	Trumbull		6	1	0.0080	0.005	0.005	0.005	0.005	0.006	0.008	0.010	0.011	0.011	0.011	0.011
		40121	OK	Pittsburg		9	1	0.0021	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.003	0.003	0.003	0.003
		41071	OR	Yamhill		10	1	0.0166	0.009	0.009	0.009	0.011	0.013	0.016	0.019	0.026	0.027	0.027	0.027
		42003	PA	Allegheny		20	2	0.0414	0.009	0.009	0.011	0.012	0.017	0.030	0.054	0.099	0.120	0.138	0.138
		42007	PA	Beaver		41	3	0.1160	0.043	0.043	0.052	0.056	0.083	0.114	0.159	0.170	0.187	0.206	0.206
		42011	PA	Berks		105	6	0.0995	0.038	0.039	0.041	0.045	0.051	0.078	0.145	0.183	0.197	0.242	0.251
		42045	PA	Delaware		10	1	0.0447	0.043	0.043	0.043	0.043	0.043	0.045	0.046	0.047	0.047	0.047	0.047
		42055	PA	Franklin		7	1	0.0447	0.043	0.043	0.043	0.043	0.043	0.045	0.046	0.046	0.046	0.046	0.046
		42063	PA	Indiana		10	1	0.0447	0.043	0.043	0.043	0.043	0.043	0.044	0.046	0.049	0.049	0.049	0.049
		42079	PA	Luzerne		6	1	0.1078	0.084	0.084	0.084	0.084	0.085	0.103	0.135	0.137	0.137	0.137	0.137
		42129	PA	Westmoreland		10	1	0.0434	0.041	0.041	0.041	0.042	0.042	0.044	0.044	0.046	0.046	0.046	0.046
		47093	TN	Knox		44	2	0.0165	0.007	0.007	0.009	0.009	0.012	0.016	0.020	0.023	0.027	0.035	0.035
		47163	TN	Sullivan		118	4	0.0554	0.030	0.030	0.033	0.035	0.039	0.045	0.060	0.100	0.125	0.134	0.168
		48085	TX	Collin		108	3	0.3101	0.048	0.051	0.070	0.085	0.120	0.217	0.469	0.682	0.753	1.189	1.262
		51770	VA	Roanoke City		10	1	0.0466	0.013	0.013	0.013	0.016	0.019	0.026	0.097	0.108	0.109	0.109	0.109
		55117	WI	Sheboygan		10	1	0.0897	0.012	0.012	0.012	0.034	0.058	0.076	0.126	0.164	0.170	0.170	0.170
		72013	PR	Arecibo (Puerto Rico)		10	1	0.1725	0.059	0.059	0.059	0.068	0.129	0.194	0.213	0.241	0.245	0.245	0.245

Table 2-14 (Continued): Distribution of 3-month moving average Pb-TSP concentrations (µg/m^3) nationwide, source-oriented monitors, 2008-2010.

Year	Season	State/ County	State	County name	Site ID	N: mo means	N sites	Mean	Min	1	5	10	25	50	75	90	95	99	max
Statistics for individual sites where overall average monthly mean ≥ national 90th percentile (2008-2010)																			
					011090003	25		0.5771	0.223	0.223	0.247	0.256	0.302	0.574	0.719	1.088	1.178	1.210	1.210
					060371405	36		0.7174	0.188	0.188	0.234	0.237	0.309	0.476	0.791	2.178	2.452	2.489	2.489
					290930016	36		0.6682	0.207	0.207	0.258	0.313	0.418	0.543	0.634	1.167	2.076	2.456	2.456
					290930021	36		0.6950	0.173	0.173	0.192	0.218	0.346	0.689	0.954	1.214	1.275	1.937	1.937
					290990004	36		1.0090	0.640	0.640	0.655	0.699	0.775	0.913	1.081	1.555	2.011	2.017	2.017
					290990015[b]	21		1.3162	0.612	0.612	0.632	0.743	0.921	1.074	1.258	2.621	2.634	2.889	2.889
					290990020[b]	29		0.6680	0.452	0.452	0.471	0.482	0.555	0.651	0.754	0.891	0.943	0.989	0.989
					290990021[b]	21		0.7317	0.429	0.429	0.435	0.507	0.547	0.685	0.900	0.999	1.013	1.141	1.141
					290999001[b]	22		0.8413	0.587	0.587	0.592	0.600	0.699	0.845	0.963	1.061	1.100	1.204	1.204
					290999005[b]	22		0.9875	0.612	0.612	0.630	0.644	0.783	0.995	1.220	1.271	1.278	1.375	1.375
					480850009[b]	36		0.6068	0.196	0.196	0.268	0.335	0.469	0.585	0.704	0.965	1.189	1.262	1.262

[a]The 3-month averages presented here were created using a simplified approach of the procedures detailed in 40 CFR part 50 appendix R and as such cannot be directly compared to the Pb NAAQS for determination of compliance with the Pb NAAQS.

[b]Sites listed in the bottom six rows of this table fall in the upper 90th percentile of the data pooled by site.

Table 2-15 Distribution of 3-month moving average Pb-TSP concentrations (µg/m^3) nationwide, non-source-oriented monitors, 2008-2010.

Year	Season	State/ County	ST	County name	Site ID	N: mo means	N sites	Mean	Min	1	5	10	25	50	75	90	95	99	max
Nationwide statistics																			
2008-2010						2,164		0.0120	0.000	0.000	0.001	0.002	0.005	0.010	0.015	0.025	0.037	0.048	0.073
2008						663		0.0130	0.000	0.000	0.001	0.002	0.005	0.011	0.016	0.027	0.040	0.050	0.055
2009						727		0.0114	0.000	0.000	0.001	0.002	0.004	0.009	0.014	0.024	0.038	0.043	0.073
2010						774		0.0118	0.000	0.000	0.001	0.001	0.005	0.010	0.016	0.025	0.035	0.047	0.057
	Winter					494		0.0113	0.000	0.001	0.001	0.002	0.005	0.009	0.014	0.023	0.037	0.050	0.055
	Spring					548		0.0119	0.000	0.000	0.001	0.002	0.005	0.009	0.015	0.025	0.036	0.050	0.073
	Summer					565		0.0121	0.000	0.000	0.001	0.002	0.005	0.010	0.016	0.026	0.037	0.046	0.053
	Fall					557		0.0126	0.000	0.000	0.001	0.002	0.005	0.011	0.017	0.027	0.037	0.048	0.057
Nationwide statistics, pooled by site																			
2008-2010							86	0.0120	0.000	0.000	0.001	0.002	0.005	0.010	0.016	0.024	0.034	0.046	0.046
2008							59	0.0127	0.001	0.001	0.002	0.003	0.005	0.011	0.016	0.024	0.043	0.050	0.050
2009							65	0.0117	0.001	0.001	0.001	0.003	0.004	0.010	0.014	0.026	0.031	0.049	0.049
2010							71	0.0118	0.000	0.000	0.001	0.001	0.005	0.010	0.017	0.022	0.028	0.045	0.045
	Winter						84	0.0118	0.000	0.000	0.001	0.002	0.005	0.010	0.015	0.025	0.036	0.048	0.048
	Spring						83	0.0118	0.000	0.000	0.001	0.002	0.004	0.010	0.015	0.025	0.034	0.059	0.059
	Summer						86	0.0118	0.000	0.000	0.001	0.002	0.005	0.009	0.016	0.023	0.037	0.043	0.043
	Fall						86	0.0126	0.000	0.000	0.001	0.002	0.005	0.011	0.016	0.026	0.030	0.046	0.046
Statistics for individual counties (2008-2010)																			
	06025		CA	Imperial		31	1	0.0165	0.007	0.007	0.008	0.011	0.013	0.017	0.021	0.023	0.023	0.023	0.023
	06037		CA	Los Angeles		218	8	0.0100	0.000	0.000	0.002	0.004	0.006	0.009	0.013	0.016	0.020	0.028	0.035
	06065		CA	Riverside		72	2	0.0078	0.002	0.002	0.004	0.005	0.007	0.008	0.010	0.011	0.011	0.011	0.011
	06071		CA	San Bernardino		69	2	0.0091	0.003	0.003	0.005	0.006	0.007	0.009	0.011	0.013	0.014	0.017	0.017
	08005		CO	Arapahoe		7	1	0.0126	0.011	0.011	0.011	0.011	0.011	0.013	0.014	0.014	0.014	0.014	0.014
	08031		CO	Denver		10	1	0.0054	0.004	0.004	0.004	0.004	0.005	0.006	0.006	0.006	0.006	0.006	0.006
	13089		GA	DeKalb		8	1	0.0035	0.003	0.003	0.003	0.003	0.003	0.004	0.004	0.004	0.004	0.004	0.004
	17031		IL	Cook		287	8	0.0196	0.010	0.010	0.010	0.010	0.012	0.017	0.025	0.033	0.038	0.047	0.051

Table 2-15 (Continued): Distribution of 3-month moving average Pb-TSP concentrations (µg/m^3) nationwide, non-source-oriented monitors, 2008-2010.

Year	Season	State/ County	ST	County name	Site ID	N: mo means	N sites	Mean	Min	1	5	10	25	50	75	90	95	99	max
	17117		IL	Macoupin		24	1	0.0101	0.010	0.010	0.010	0.010	0.010	0.010	0.010	0.011	0.011	0.011	0.011
	17119		IL	Madison		36	1	0.0188	0.010	0.010	0.010	0.011	0.014	0.016	0.022	0.036	0.036	0.039	0.039
	17143		IL	Peoria		36	1	0.0105	0.010	0.010	0.010	0.010	0.010	0.010	0.011	0.012	0.012	0.013	0.013
	17163		IL	Saint Clair		36	1	0.0204	0.012	0.012	0.012	0.014	0.016	0.020	0.024	0.029	0.033	0.036	0.036
	18089		IN	Lake		36	1	0.0149	0.007	0.007	0.007	0.007	0.010	0.014	0.018	0.024	0.032	0.037	0.037
	18097		IN	Marion		33	1	0.0056	0.003	0.003	0.003	0.003	0.004	0.005	0.007	0.009	0.010	0.011	0.011
	18163		IN	Vanderburgh		31	2	0.0047	0.002	0.002	0.003	0.003	0.004	0.005	0.005	0.006	0.007	0.007	0.007
	25025		MA	Suffolk		24	2	0.0093	0.005	0.005	0.006	0.006	0.008	0.009	0.011	0.013	0.015	0.016	0.016
	26081		MI	Kent		10	1	0.0055	0.004	0.004	0.004	0.005	0.005	0.006	0.006	0.006	0.006	0.006	0.006
	26163		MI	Wayne		32	2	0.0119	0.004	0.004	0.004	0.005	0.005	0.012	0.017	0.021	0.023	0.024	0.024
	27017		MN	Carlton		10	1	0.0000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	27037		MN	Dakota		112	5	0.0036	0.000	0.000	0.001	0.001	0.001	0.003	0.005	0.007	0.012	0.013	0.015
	27053		MN	Hennepin		124	4	0.0033	0.000	0.001	0.001	0.001	0.002	0.003	0.004	0.006	0.006	0.015	0.016
	27075		MN	Lake		8	1	0.0000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	27123		MN	Ramsey		65	3	0.0061	0.001	0.001	0.001	0.001	0.002	0.005	0.008	0.014	0.016	0.017	0.017
	27137		MN	Saint Louis		70	2	0.0016	0.000	0.000	0.000	0.000	0.001	0.001	0.002	0.004	0.004	0.005	0.005
	27163		MN	Washington		70	3	0.0017	0.000	0.000	0.000	0.000	0.001	0.001	0.003	0.004	0.004	0.005	0.005
	29097		MO	Jasper		10	1	0.0135	0.009	0.009	0.009	0.011	0.012	0.014	0.015	0.016	0.017	0.017	0.017
	29187		MO	Saint Francois		21	2	0.0337	0.011	0.011	0.012	0.012	0.027	0.035	0.042	0.048	0.053	0.054	0.054
	29189		MO	Saint Louis		33	1	0.0243	0.005	0.005	0.005	0.006	0.007	0.008	0.050	0.050	0.050	0.055	0.055
	36047		NY	Kings		24	1	0.0131	0.011	0.011	0.011	0.011	0.012	0.013	0.014	0.016	0.018	0.019	0.019
	39017		OH	Butler		30	1	0.0055	0.003	0.003	0.004	0.004	0.005	0.006	0.006	0.007	0.007	0.008	0.008
	39029		OH	Columbiana		105	3	0.0148	0.005	0.005	0.007	0.008	0.010	0.013	0.017	0.021	0.028	0.054	0.057
	39035		OH	Cuyahoga		105	3	0.0144	0.005	0.006	0.006	0.008	0.010	0.013	0.018	0.023	0.027	0.033	0.035
	39049		OH	Franklin		36	1	0.0092	0.005	0.005	0.005	0.005	0.008	0.010	0.011	0.011	0.012	0.012	0.012
	39143		OH	Sandusky		10	1	0.0052	0.004	0.004	0.004	0.004	0.005	0.005	0.006	0.006	0.006	0.006	0.006
	39167		OH	Washington		48	2	0.0047	0.002	0.002	0.002	0.003	0.004	0.004	0.006	0.007	0.007	0.008	0.008
	40115		OK	Ottawa		12	2	0.0128	0.005	0.005	0.005	0.006	0.010	0.014	0.016	0.018	0.019	0.019	0.019
	42003		PA	Allegheny		36	1	0.0101	0.000	0.000	0.000	0.000	0.007	0.012	0.014	0.016	0.018	0.025	0.025
	42021		PA	Cambria		23	1	0.0459	0.040	0.040	0.040	0.040	0.040	0.041	0.046	0.069	0.070	0.073	0.073
	42045		PA	Delaware		14	1	0.0427	0.040	0.040	0.040	0.040	0.040	0.042	0.045	0.046	0.047	0.047	0.047
	42101		PA	Philadelphia		22	1	0.0214	0.013	0.013	0.014	0.014	0.018	0.022	0.025	0.029	0.029	0.030	0.030

Table 2-15 (Continued): Distribution of 3-month moving average Pb-TSP concentrations (µg/m^3) nationwide, non-source-oriented monitors, 2008-2010.

Year	Season	State/ County	ST	County name	Site ID	N: mo means	N sites	Mean	Min	1	5	10	25	50	75	90	95	99	max
	42129		PA	Westmoreland		24	1	0.0417	0.037	0.037	0.040	0.040	0.040	0.041	0.043	0.046	0.047	0.048	0.048
	48061		TX	Cameron		33	1	0.0042	0.002	0.002	0.003	0.003	0.004	0.004	0.005	0.005	0.006	0.006	0.006
	48141		TX	El Paso		56	3	0.0212	0.014	0.014	0.014	0.015	0.016	0.018	0.023	0.038	0.040	0.040	0.040
	48201		TX	Harris		30	1	0.0051	0.004	0.004	0.004	0.004	0.005	0.005	0.006	0.006	0.007	0.007	0.007
	48479		TX	Webb		23	1	0.0121	0.006	0.006	0.007	0.007	0.008	0.010	0.016	0.021	0.022	0.026	0.026
	49035		UT	Salt Lake		10	1	0.0145	0.007	0.007	0.007	0.007	0.008	0.011	0.016	0.032	0.036	0.036	0.036
Statistics for individual sites where overall average monthly mean ≥ national 90th percentile (2008-2010)																			
					170310022	36		0.0335	0.016	0.016	0.018	0.026	0.028	0.032	0.038	0.047	0.048	0.051	0.051
					170310026	36		0.0281	0.018	0.018	0.019	0.022	0.023	0.026	0.032	0.038	0.043	0.046	0.046
					170316003	36		0.0245	0.015	0.015	0.015	0.017	0.020	0.025	0.028	0.031	0.035	0.036	0.036
					291870006[b]	10		0.0412	0.017	0.017	0.017	0.026	0.035	0.043	0.048	0.054	0.054	0.054	0.054
					291870007[b]	11		0.0268	0.011	0.011	0.011	0.012	0.012	0.028	0.035	0.036	0.041	0.041	0.041
					291892003[b]	33		0.0243	0.005	0.005	0.005	0.006	0.007	0.008	0.050	0.050	0.050	0.055	0.055
					420210808[b]	23		0.0459	0.040	0.040	0.040	0.040	0.040	0.041	0.046	0.069	0.070	0.073	0.073
					420450002[b]	14		0.0427	0.040	0.040	0.040	0.040	0.040	0.042	0.045	0.046	0.047	0.047	0.047
					421290007[b]	24		0.0417	0.037	0.037	0.040	0.040	0.040	0.041	0.043	0.046	0.047	0.048	0.048

[a]The 3-month averages presented here were created using a simplified approach of the procedures detailed in 40 CFR part 50 appendix R and as such cannot be directly compared to the Pb NAAQS for determination of compliance with the Pb NAAQS.

[b]Sites listed in the bottom six rows of this table fall in the upper 90th percentile of the data pooled by site.

Table 2-16 Distribution of annual 1-month site maxima TSP Pb concentrations (µg/m^3) nationwide, source-oriented monitors, 2008-2010.

Year	Site ID – year	N (sites)	Mean	Min	1	5	10	25	50	75	90	95	99	max
Nationwide statistics														
2008-2010		111	0.5003	0.003	0.006	0.016	0.032	0.066	0.156	0.575	1.530	2.416	4.225	4.440
2008		47	0.8138	0.012	0.012	0.052	0.057	0.096	0.320	0.850	2.557	3.123	4.440	4.440
2009		54	0.4486	0.016	0.016	0.022	0.050	0.090	0.170	0.618	1.280	1.623	2.438	2.438
2010		101	0.3105	0.003	0.006	0.008	0.024	0.054	0.142	0.347	0.854	1.117	1.576	1.828
Annual site max 1-month means >= national 90th percentile (2008-20010)														
	060371405-2008		2.8800											
	180350009-2008		4.4400											
	290930016-2008		4.2252											
	290930021-2008		2.5566											
	290930021-2009		2.4380											
	290990004-2008		2.4156											
	290990004-2009		1.5599											
	290990004-2010		1.5762											
	290990011-2008		1.5295											
	290990015[a]-2008		3.1228											
	290990020[a]-2008		2.2204											
	290990021[a]-2008		1.5528											
	290999001[a]-2009		1.6228											
	290999001[a]-2010		1.5576											
	290999005[a]-2009		1.9850											
	290999005[a]-2010		1.8278											
	480850009[a]-2008		1.5640											

[a]Sites listed in the bottom eight rows of this table fall in the upper 90th percentile of the data pooled by site.

Table 2-17 Distribution of annual 1-month site maxima TSP Pb concentrations (µg/m^3) nationwide, non-source-oriented monitors, 2008-2010.

Year	Site ID – year	N (sites)	Mean	Min	1	5	10	25	50	75	90	95	99	max
Nationwide statistics														
2008-2010		88	0.0284	0.000	0.000	0.004	0.006	0.010	0.020	0.041	0.057	0.070	0.136	0.136
2008		59	0.0232	0.004	0.004	0.005	0.006	0.010	0.016	0.033	0.053	0.058	0.066	0.066
2009		66	0.0210	0.003	0.003	0.005	0.006	0.008	0.014	0.026	0.040	0.056	0.128	0.128
2010		73	0.0233	0.000	0.000	0.002	0.004	0.008	0.015	0.029	0.049	0.065	0.136	0.136
Annual site max 1-month means >= national 90th percentile (2008-2010)														
	170310022-2009		0.0700											
	170310022-2010		0.0620											
	171193007-2008		0.0660											
	291870006[a]-2010		0.0894											
	291892003[a]-2008		0.0660											
	390290019[a]-2010		0.1360											
	390290022[a]-2010		0.0652											
	420210808[a]-2008		0.0583											
	420210808[a]-2009		0.1280											
	481410002[a]-2010		0.0870											
	481410033[a]-2009		0.0570											

[a]Sites listed in the bottom eight rows of this table fall in the upper 90th percentile of the data pooled by site.

Table 2-18 Distribution of annual 3-month site maxima Pb-TSP concentrations (µg/m^3) nationwide, source-oriented monitors, 2008-2010.

Year	Site ID – year	N (sites)	Mean	Min	1	5	10	25	50	75	90	95	99	max
Nationwide statistics[a]														
2008-2010		106	0.3605	0.003	0.005	0.016	0.023	0.047	0.109	0.378	1.204	1.937	2.489	2.889
2007		47	0.5831	0.009	0.009	0.038	0.043	0.085	0.242	0.815	2.017	2.456	2.889	2.889
2008		54	0.3611	0.012	0.012	0.017	0.035	0.060	0.121	0.467	1.079	1.258	2.070	2.070
2009		96	0.2112	0.003	0.003	0.011	0.021	0.046	0.091	0.262	0.630	0.865	1.375	1.375
Annual site max 3-month means >= national 90th percentile (2008-2010)														
	011090003-2008		1.2100											
	060371405-2008		2.4890											
	120571066-2008		1.7700											
	180350009-2008		2.1630											
	290930016[b]-2008		2.4560											
	290930016[b]-2009		2.0700											
	290930021[b]-2009		1.9370											
	290990004[b]-2008		2.0170											
	290990015[b]-2008		2.8890											
	290999001[b]-2009		1.2040											
	290999005[b]-2009		1.2580											
	290999005[b]-2010		1.3750											
	480850009[b]-2008		1.2620											

[a]The 3-month averages presented here were created using a simplified approach of the procedures detailed in 40 CFR part 50 appendix R and as such cannot be directly compared to the Pb NAAQS for determination of compliance with the Pb NAAQS.
[b]Sites listed in the bottom nine rows of this table fall in the upper 90th percentile of the data pooled by site.

Table 2-19 Distribution of annual 3-month site maxima Pb-TSP concentrations (µg/m^3) nationwide, non-source-oriented monitors, 2008-2010.

Year	Site ID – year	N (sites)	Mean	Min	1	5	10	25	50	75	90	95	99	max
Nationwide statistics[a]														
2008-2010		86	0.0198	0.000	0.000	0.002	0.004	0.007	0.015	0.028	0.044	0.051	0.073	0.073
2008		59	0.0176	0.002	0.002	0.004	0.005	0.007	0.014	0.024	0.039	0.048	0.055	0.055
2009		65	0.0162	0.002	0.002	0.003	0.004	0.006	0.013	0.021	0.038	0.041	0.073	0.073
2010		71	0.0171	0.000	0.000	0.001	0.002	0.006	0.013	0.024	0.037	0.047	0.057	0.057
Annual site max 3-month means >= national 90th percentile (2008-2010)														
	170310022-2008		0.0480											
	170310022-2009		0.0470											
	170310022-2010		0.0510											
	170310026[b]-2008		0.0460											
	291870006[b]-2010		0.0540											
	291892003[b]-2008		0.0550											
	390290019[b]-2010		0.0570											
	390290022[b]-2010		0.0440											
	420210808[b]-2008		0.0490											
	420210808[b]-2009		0.0730											
	420450002[b]-2010		0.0470											
	421290007[b]-2008		0.0480											

[a]The 3-month averages presented here were created using a simplified approach of the procedures detailed in 40 CFR part 50 appendix R and as such cannot be directly compared to the Pb NAAQS for determination of compliance with the Pb NAAQS.

[b] Sites listed in the bottom nine rows of this table fall in the upper 90th percentile of the data pooled by site.

Concentrations of Pb Measured using PM_{10} Monitors (for Concentrations and Trends)

Figure 2-38 displays maximum 3-month averages for Pb-PM_{10} concentrations for 36 counties in which measurements were obtained. Among the 36 counties in which PM_{10} monitoring was conducted, only one county, Gila County, AZ, reported concentrations above 0.076 µg/m^3. Three other counties reported concentrations greater than 0.016 µg/m^3: Wayne County, MI, Boyd County, KY, and the county of St. Louis City, MO.

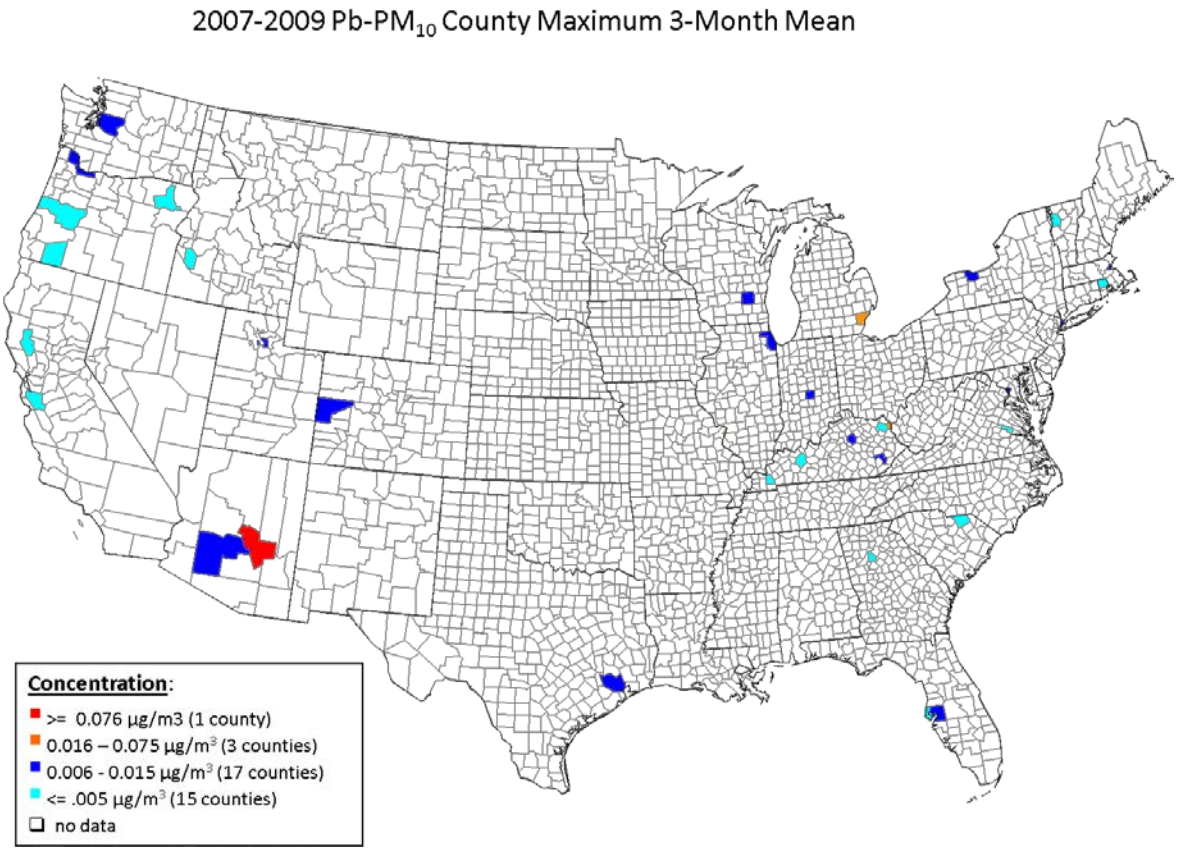


Figure 2-38 Highest county-level Pb-PM_{10} concentrations (µg/m^3), maximum 3-month average, 2007-2009.

Concentrations of Pb Measured using $PM_{2.5}$ Monitors (for Speciation Concentrations and Trends)

Figure 2-39 displays maximum 3-month average county-level data for Pb in $PM_{2.5}$ concentrations for 323 counties in which $PM_{2.5}$ measurements were obtained for speciation in the CSN and IMPROVE networks. The data presented here are not compared to the NAAQS because $PM_{2.5}$ monitors are not deployed for the purpose of evaluating compliance for the NAAQS. Among the 323 counties in which $PM_{2.5}$ monitoring was conducted, only eleven counties reported concentrations greater than 0.016 µg/m^3: Jefferson, AL, San Bernardino, CA, Imperial, CA, Wayne, MI, Jefferson, MO, Erie, NY, Lorain, OH, Allegheny, PA, Berks, PA, Davidson, TN, and El Paso, TX.

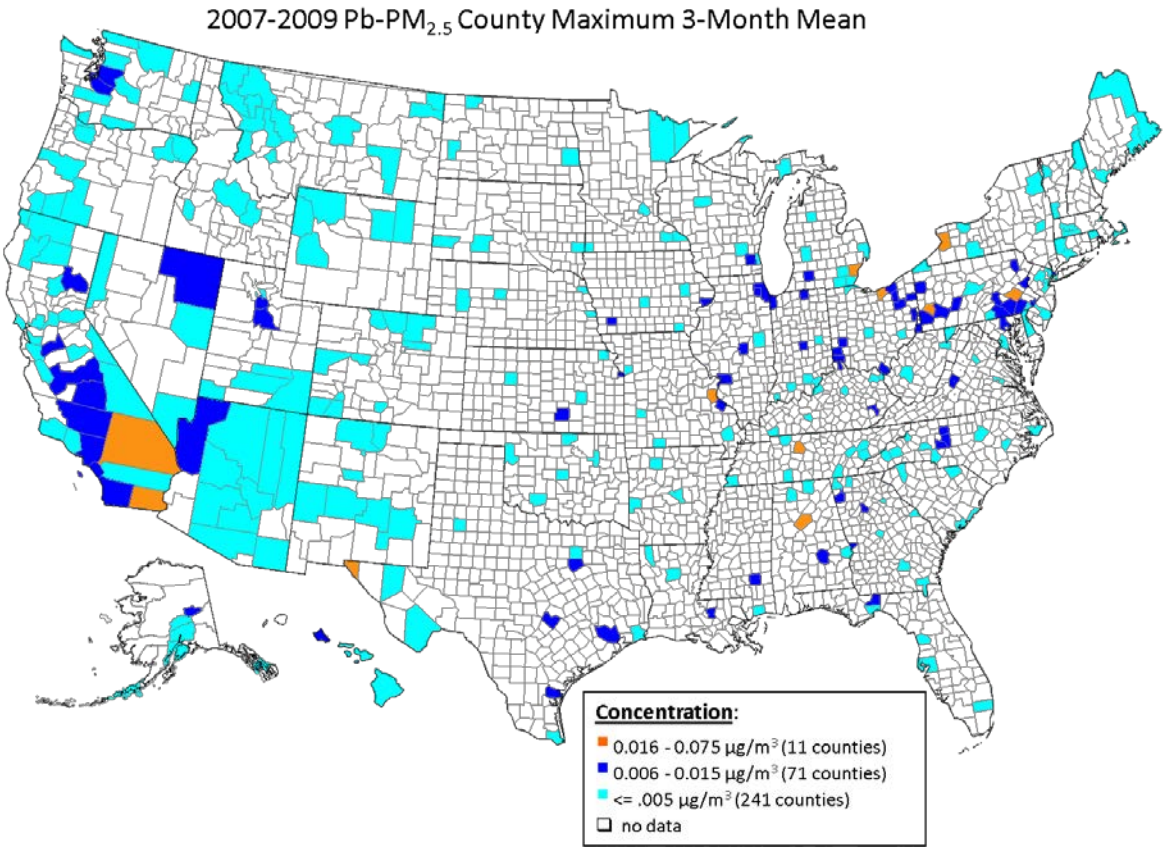


Figure 2-39 Highest county-level Pb-$PM_{2.5}$ concentrations (µg/m^3), maximum 3-month average, 2007-2009.

2.8.2 Intra-urban Variability

Intra-urban variability in Pb concentrations reported to AQS was described in detail for Los Angeles County, CA (Los Angeles), Hillsborough and Pinellas Counties, FL (Tampa), Cook County, IL (Chicago), Jefferson County, MO (Herculaneum), Cuyahoga County, OH (Cleveland), and Sullivan County, TN (Bristol) were selected for this assessment to illustrate the variability in Pb concentrations measured across different metropolitan regions with varying Pb source characteristics. Four of the counties encompass large cities (Los Angeles, Tampa, Chicago, and Cleveland). All six counties contain source-oriented monitors. Maps and wind roses (graphs representing wind direction and wind speed at a location) are presented in this Chapter 2 Appendix for each of the six urban areas. Additionally, annual and seasonal box plots of the Pb concentration distributions and intra-monitor correlation tables are presented to illustrate the level of variability throughout each urban area.

Maps of six areas (Los Angeles County, CA; Hillsborough/Pinellas Counties, FL; Cook County, IL; Jefferson County, MO; Cuyahoga County, OH; and Sullivan County, TN) are shown to illustrate the location of all Pb monitors meeting the inclusion criteria. Wind roses for each season are also provided to help put the source concentration data in context. Letters on the maps identify the individual monitor locations and correspond with the letters provided in the accompanying concentration box plots and pair-wise monitor comparison tables. The box plots for each monitor include the annual and seasonal concentration median and interquartile range with whiskers extending from the 5th to the 95th percentile. Data from 2008-2010 were used to generate the box plots, which are stratified by season as follows: 1 = winter (December-February), 2 = spring (March-May), 3 = summer (June-August), and 4 = fall (September-November). The comparison tables include the Pearson correlation coefficient (R), Spearman rank-ordered correlation coefficient (ρ), the 90th percentile of the absolute difference in concentrations (P90) in μg/m^3, the coefficient of divergence (*COD*) and the straight-line distance between monitor pairs (d) in km. The *COD* provides an indication of the variability across the monitoring sites within each county and is defined as follows:

$$COD_{jk} = \sqrt{\frac{1}{p}\sum_{i=1}^{p}\left(\frac{X_{ij} - X_{ik}}{X_{ij} + X_{ik}}\right)^2}$$

Equation 2A-1

where X_{ij} and X_{ik} represent the observed hourly concentrations for time period *i* at sites *j* and *k*, and *p* is the number of paired hourly observations. A *COD* of 0 indicates there are

no differences between concentrations at paired sites (spatial homogeneity), while a *COD* approaching 1 indicates extreme spatial heterogeneity.

In certain cases, the information contained in these figures and tables should be used with some caution since many of the reported concentrations for the years 2008-2010 are near or below the analysis method's stated method detection limit (MDL). The MDL is generally taken as 0.01 because it is the upper value of the range of MDLs reported for atomic absorption (AA) and Emissions Spectra ICAP methods, which were the two methods reported in the AQS to have been used for analysis of FRM samples (Rice, 2007). Generally, data are reported to the hundredth place, so this assumption is reasonable. The approximate percentage of data below the MDL (to the nearest 5%) is provided for each site along with box plots of seasonal Pb concentration at monitors within each urban area studied.

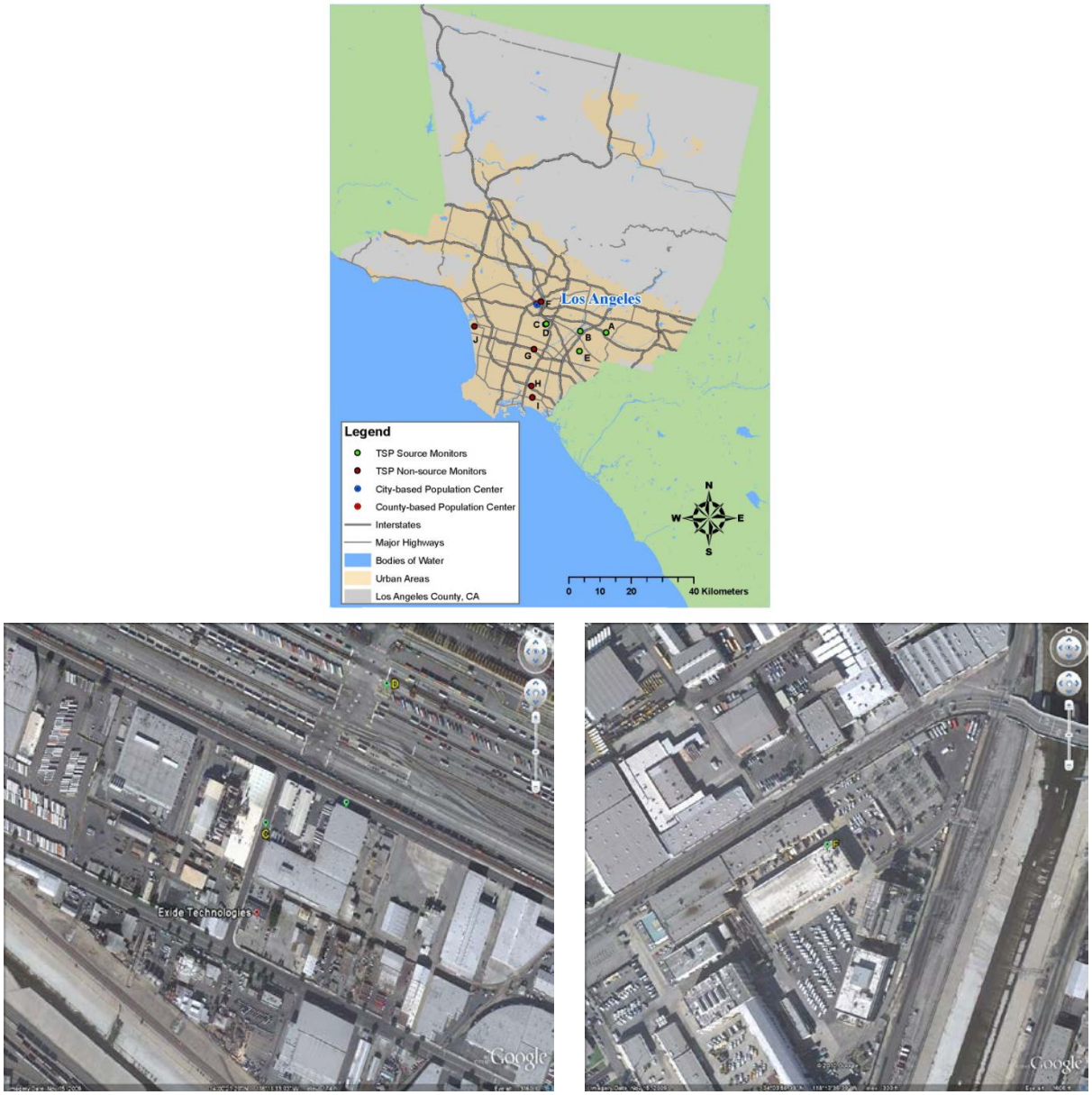
Figure 2-40 illustrates Pb monitor locations within Los Angeles County, CA. Ten monitors are located within Los Angeles County, five of which were source-oriented and the other five were non-source-oriented monitors. Monitor A was located immediately downwind of the Quemetco battery recycling facility in the City of Industry, CA. This source was estimated to produce 0.32 tons of Pb/year (U.S. EPA, 2008c). Monitor C was sited in a street canyon just upwind of the Exide Pb recycling facility, which was estimated to produce 2.0 tons of Pb/year (U.S. EPA, 2008c). Monitor D was situated slightly northwest of the same Pb recycling facility. It is still in relatively close proximity but not downwind on most occasions. Monitor B was located 12 km downwind of the Exide facility. Monitor E was located nearby the Trojan Battery recycling facility, which emitted 0.79 tons Pb/year (U.S. EPA, 2008c). Location of the non-source-oriented monitors varied. Monitor F was positioned on a roof top 60 meters away from a 4-lane arterial road and 100 meters from of a railroad. Monitor G was located on a rooftop approximately 20 meters from an 8-lane arterial road, and monitor H was positioned at the curbside of a four-lane road roughly 650 meters north of that road's junction with Interstate I-405. Monitor I was sited in a parking lot roughly 80 meters from a four-lane road, and monitor J was located approximately 130 meters south of a 4-lane highway. Figure 2-41 displays seasonal wind roses for Los Angeles County. In spring, summer, and fall, the predominant winds come from the west-southwest. During winter, wind direction varies with a portion from the west-southwest and the remainder from the east. The highest winds during winter come more frequently from the west-southwest.

The maps shown in Figure 2-40 for source-oriented monitors A-E illustrate the different conditions captured by the monitors; this informs analysis of the seasonal and year-round concentrations reported in Figure 2-42. The average annual concentration at monitor A was 0.074 $\mu g/m^3$. The 95th percentile exceeded the level of the NAAQS in the spring

(0.16 μg/m^3) and summer (0.18 μg/m^3). Monitor C reported the highest concentrations in Los Angeles County, with a year-round mean of 0.68 μg/m^3. Given the position of this monitor with respect to the Exide facility, there is the potential for recirculation of fugitive Pb emissions in the air sampled by that monitor. The average annual Pb concentration at monitor D was 0.12 μg/m^3, and the 75th percentile of year-round data exceeded the level of the NAAQS; in spring, the 70th percentile exceeded 0.15 μg/m^3. Monitor B reported the lowest values among the source-oriented monitors with an average annual concentration of 0.013 μg/m^3. Note that 75% of reported values were below the MDL for this site, and no data from this site exceeded the level of the NAAQS. The annual average concentration at monitor E was 0.068 μg/m^3, and the 95th percentile of concentration was 0.17 μg/m^3.

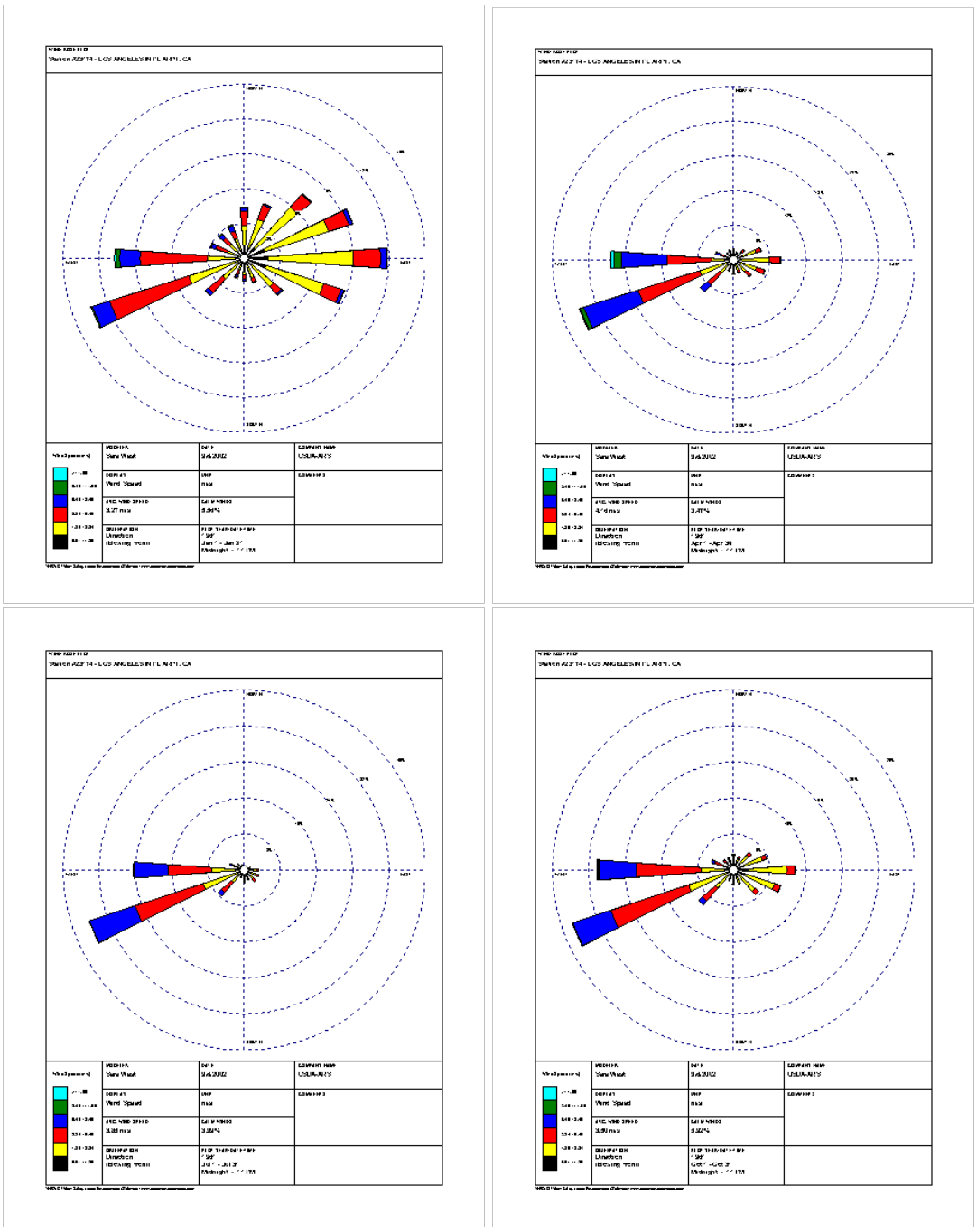
The non-source-oriented monitors located at sites F-J all recorded low concentrations, with average values ranging from 0.004 to 0.018 μg/m^3 (Figure 2-42). The highest average year-round concentrations were recorded at site F. The 95th percentiles at these sites ranged from 0.01 to 0.04 μg/m^3. There is much less certainty in the data recorded at the non-source-oriented sites, because 45-95% of the data from these monitors were below the MDL. Additionally, only one of the non-source-oriented monitors (monitor H) was positioned at roadside, and none of the non-source-oriented monitors were located at the side of a major highway.

Intersampler correlations (Table 2-20), illustrate that Pb has high intra-urban spatial variability. For the source-oriented monitors, the highest correlation ($R = 0.59$, $\rho = 0.57$) occurred for monitors C and D, which covered the same site. Because monitor D was slightly farther from the Exide source and slightly upstream of the predominant wind direction, the signal it received from the source site was correspondingly lower. Hence, the correlation between these sites was moderate despite their relatively close proximity. In general, low or even negative correlations were observed between the source-oriented and non-source-oriented monitors. The exception to this was the Spearman-ranked correlation between source-oriented monitor B and non-source-oriented monitor F, with $\rho = 0.74$. Pearson correlation was much lower for this pair ($R = 0.33$). Monitors B and F are roughly 16 km apart, whereas monitor B is only 12 km from monitors D and C, 8 km from monitor E, and 6 km from monitor A. It is possible that monitors B and F both captured a source that was either longer in range or more ubiquitous and so would have been obscured by the stronger source signals at sites A, C, D, and E. Comparisons between the non-source-oriented monitors revealed moderate correlation between sites (G to J [$R = 0.29$ to 0.71, $\rho = 0.37$ to 0.65]). Sites G, H, I and J are all located in the southwestern quadrant of Los Angeles. It is possible that they are also exposed to a ubiquitous source that produces a common signal at these four sites.



Note: Monitor locations are denoted by green markers, and source locations are denoted by red markers. Top: view of all Pb FRM monitors in Los Angeles County. Bottom left: Close up of the industrial site near Monitors C and D. Bottom right: Close up of the populated area captured by Monitor F.

Figure 2-40 Pb TSP monitor and source locations within Los Angeles County, CA (06-037), 2007-2009.



Note: Clockwise from top left: January, April, July, and October. Note that the wind percentages vary from month to month.
Source: NRCS (2011).

Figure 2-41 Wind roses for Los Angeles County, CA, from meteorological data at the Los Angeles International Airport, 1961-1990.

Site	A	B	C	D	E	F	G	H	I	J
SITE ID	06-037-1404	06-037-1602	06-037-1405	06-037-1406	06-037-1403	06-037-1103	06-037-1301	06-037-4002	06-037-4004	06-037-5005
MEAN	0.074	0.013	0.68	0.12	0.068	0.018	0.015	0.0083	0.0087	0.0040
SD	0.040	0.017	1.0	0.092	0.052	0.011	0.012	0.0068	0.0069	0.0064
OBS	66	112	617	242	128	121	108	120	117	109
% BELOW MDL	0	75	0	0	0	45	65	85	85	95
Source orientation	Source	Source	Source	Source	Source	Non-source	Non-source	Non-source	Non-source	Non-source

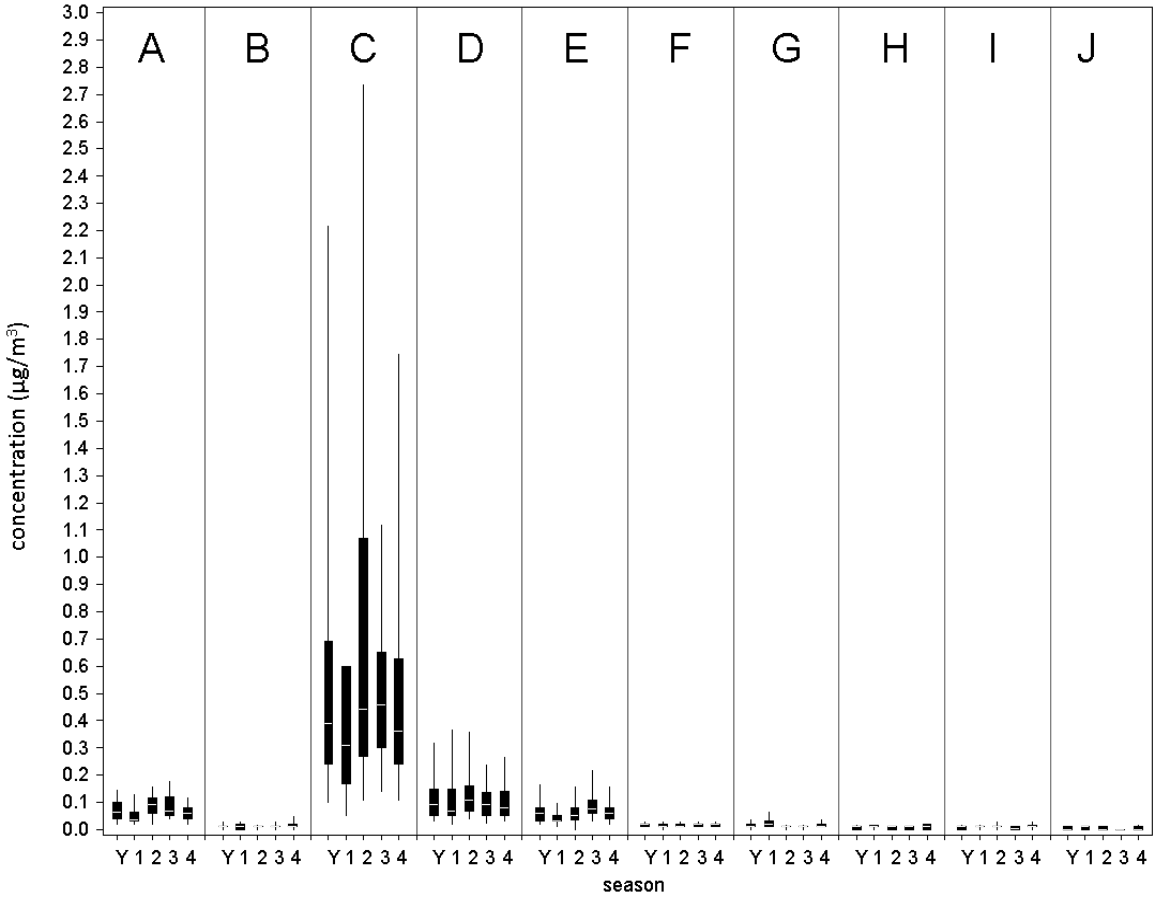


Figure 2-42 Box plots of annual and seasonal 24-h Pb TSP concentrations (µg/m^3) from source-oriented and non-source-oriented monitors within Los Angeles County, CA (06-037), 2007-2009.

Table 2-20 Comparisons between Pb TSP concentrations from source-oriented and non-source-oriented monitors within Los Angeles County, CA (06-037), 2007-2009.

			A	B	C	D	E	F	G	H	I	J
			Source	Source	Source	Source	Source	Non-Source	Non-Source	Non-Source	Non-Source	Non-Source
A	Source	R	1.00	-0.04	0.14	0.10	0.17	0.03	0.00	-0.08	-0.07	-0.27
		ρ	1.00	0.16	0.10	0.08	0.27	-0.15	0.00	0.14	-0.02	-0.09
		P90	0.00	0.08	0.49	0.10	0.10	0.08	0.06	0.08	0.08	0.08
		COD	0.00	0.63	0.64	0.31	0.34	0.57	0.57	0.79	0.77	0.85
B	Source	R		1.00	0.06	0.17	-0.06	0.33	0.29	0.40	0.22	0.20
		ρ		1.00	0.05	0.05	0.07	0.74	0.12	0.28	0.11	0.10
		P90		0.00	3.59	0.25	0.10	0.02	0.02	0.01	0.02	0.02
		COD		0.00	0.96	0.84	0.71	0.46	0.48	0.61	0.60	0.81
C	Source	R			1.00	0.59	0.08	0.12	0.24	0.28	0.18	0.08
		ρ			1.00	0.57	0.03	-0.08	0.26	0.28	0.20	0.13
		P90			0.00	1.76	2.14	3.59	4.22	3.59	3.59	3.92
		COD			0.00	0.68	0.77	0.95	0.96	0.98	0.98	0.99
D	Source	R				1.00	0.18	0.33	0.09	0.32	0.20	0.03
		ρ				1.00	0.12	0.17	0.11	0.24	0.21	0.07
		P90				0.00	0.17	0.24	0.25	0.25	0.25	0.25
		COD				0.00	0.42	0.78	0.80	0.89	0.89	0.95
E	Source	R					1.00	0.05	0.07	0.00	0.09	-0.07
		ρ					1.00	0.13	0.06	0.24	0.07	0.18
		P90					0.00	0.10	0.10	0.11	0.11	0.11
		COD					0.00	0.61	0.64	0.78	0.79	0.90

Table 2-20 (Continued): Comparisons between Pb TSP concentrations from source-oriented and non-source-oriented monitors within Los Angeles County, CA (06-037), 2007-2009.

			A	B	C	D	E	F	G	H	I	J
			Source	Source	Source	Source	Source	Non-Source	Non-Source	Non-Source	Non-Source	Non-Source
F	**Non-Source**	R						1.00	0.10	0.43	0.34	0.21
		ρ						1.00	0.02	0.19	0.09	0.09
		P90						0.00	0.02	0.02	0.02	0.02
		COD						0.00	0.39	0.61	0.58	0.82
G	**Non-Source**	R							1.00	0.71	0.55	0.54
		ρ							1.00	0.65	0.39	0.38
		P90							0.00	0.01	0.02	0.02
		COD							0.00	0.54	0.61	0.85
H	**Non-Source**	R								1.00	0.60	0.51
		ρ								1.00	0.51	0.40
		P90								0.00	0.01	0.01
		COD								0.00	0.55	0.77
I	**Non-Source**	R									1.00	0.29
		ρ									1.00	0.37
		P90									0.00	0.01
		COD									0.00	0.78
J	**Non-Source**	R										1.00
		ρ										1.00
		P90										0.00
		COD										0.00

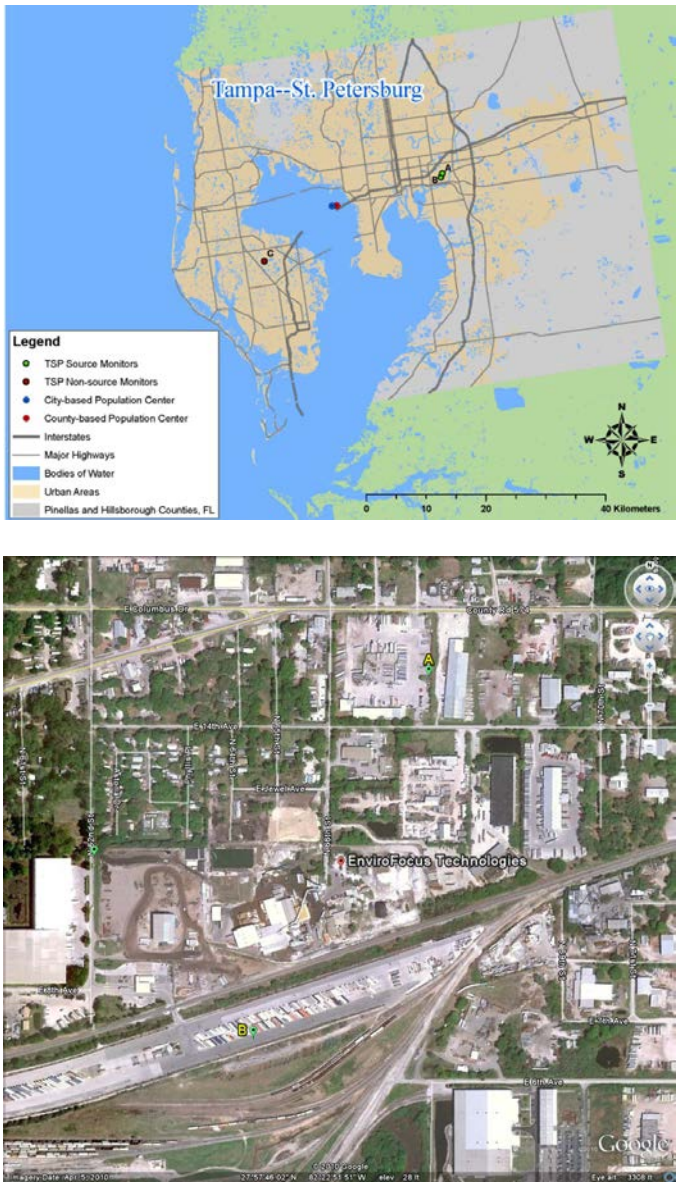
Each comparison contains (in order): Pearson rank-order correlation (R), Spearman rank-order correlation (p), the difference between the 90th and 10th percentile data (P90), and the coefficient of divergence (*COD*).

Figure 2-43 illustrates Pb monitor locations within Hillsborough and Pinellas Counties in FL, which comprise the greater Tampa-St. Petersburg metropolitan area. Two source-oriented monitors (A and B) were located within Hillsborough County, and one non-source-oriented monitor (C) was located in Pinellas County. Monitor A was located 360 meters north-northeast of the EnviroFocus Technologies battery recycling facility, which produced 1.3 tons/year (U.S. EPA, 2008d), and monitor B was located 320 meters southwest of the same facility. Monitor C was located next to a two-lane road in Pinellas Park, FL.

Figure 2-44 displays seasonal wind roses for the Tampa-St. Petersburg metropolitan area. These wind roses suggest shifting wind directions throughout the winter, spring, and summer. During the winter, the highest winds came from the north and northeast with little influence from the west and southwest. During spring and summer, easterly and westerly winds were evident from the wind rose, with winds from the west being slightly higher in wind speed. During autumn, winds came predominantly from the northeast with little signal from the west or south.

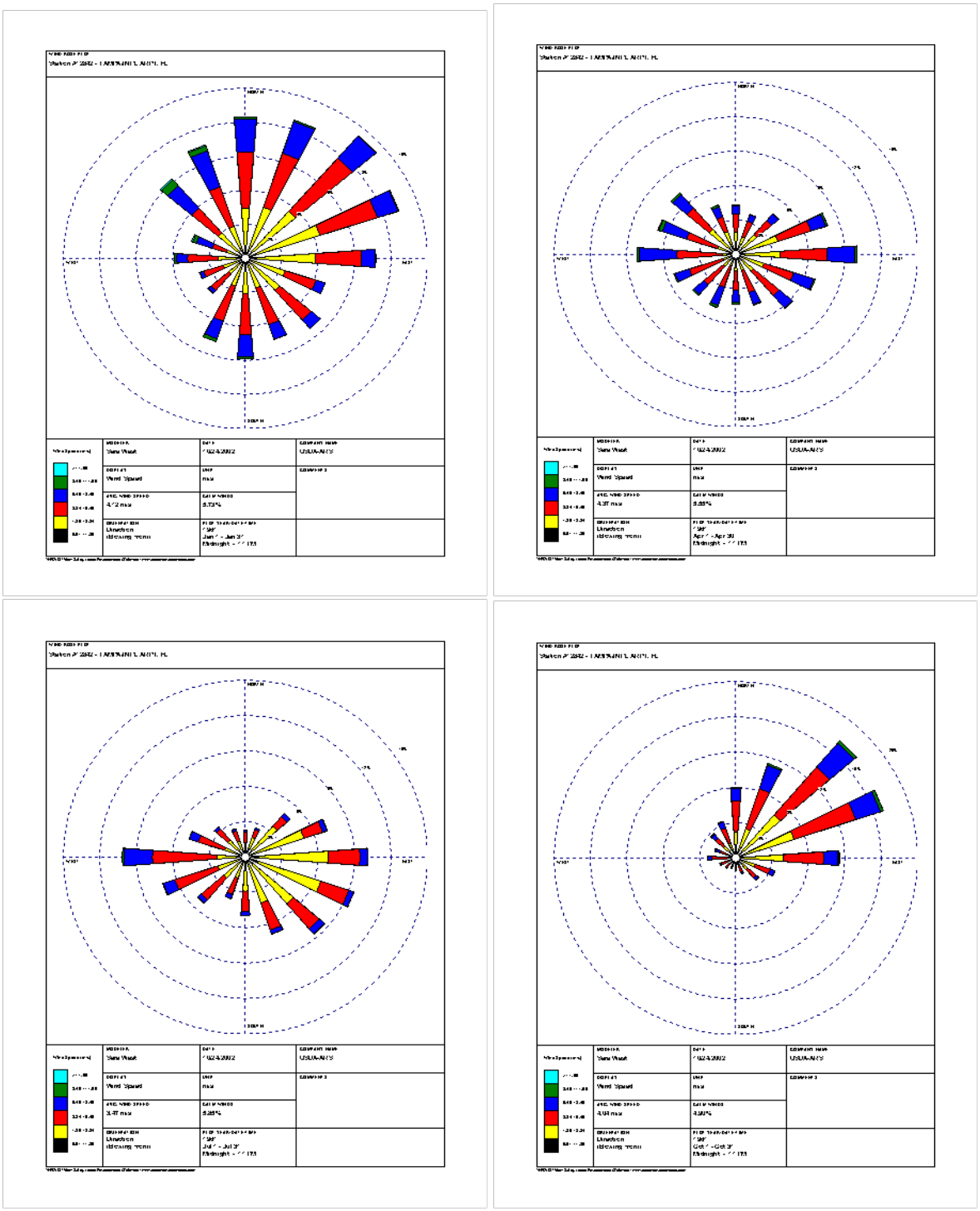
Seasonal and year-round concentrations are reported for Hillsborough and Pinellas Counties in Figure 2-45. The average annual concentration at monitor A was 0.15 $\mu g/m^3$, and the 95th percentile was 0.70 $\mu g/m^3$. During winter, the 60th percentile of the data met the level of the NAAQS. At this site, the highest concentrations occurred during summer, which corresponded to the time when westerly winds were stronger. Concentration data at monitor B were much higher, with an annual average of 0.45 $\mu g/m^3$ and a 95th percentile of 1.9 $\mu g/m^3$. Annually, the 55th percentile exceeded the level of the NAAQS, and in autumn the 45th percentile exceeded the NAAQS. The highest concentrations occurred in autumn, coinciding with the time when winds blew from the northeast, when monitor B was most often downwind of the battery recycling facility. The non-source-oriented monitor C always reported concentrations of 0.0 $\mu g/m^3$. This is likely related to its location next to a quiet road in a small city.

Intersampler correlations, shown in Table 2-21, illustrate that Pb has high intra-urban spatial variability. The source-oriented monitors were anticorrelated ($R = -0.09$, $\rho = -0.08$). This was likely related to the fact that they were designated to monitor the same source and were downwind of the source at different times.



Top: view of all Pb FRM monitors in Hillsborough and Pinellas Counties.
Bottom: Close up of industrial site around monitors A and B.

Figure 2-43 Pb TSP monitor locations within Hillsborough and Pinellas Counties, FL (12-057 and 12-103), 2007-2009.



Note: Clockwise from top left: January, April, July, and October. Note that wind percentages vary from month to month.
Source: NRCS (2011).

Figure 2-44 Wind roses for Hillsborough/Pinellas Counties, FL, obtained from meteorological data at Tampa International Airport, 1961-1990.

Site	A	B	C
SITE ID	12-057-1073	12-057-1066	12-103-3005
MEAN	0.15	0.45	0.00
SD	0.27	1.08	0.00
OBS	154	155	58
% BELOW MDL	20	5	95
Source orientation	Source	Source	Non-source

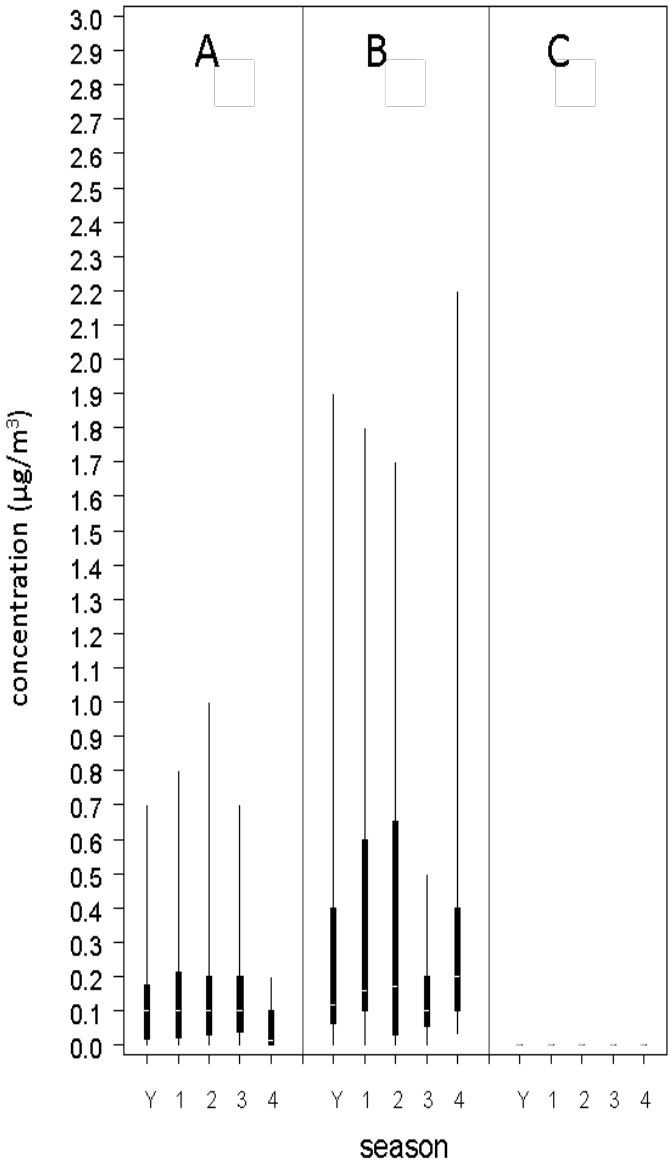


Figure 2-45 Box plots of annual and seasonal 24-h Pb TSP concentrations (µg/m^3) from source-oriented and non-source-oriented monitors within Hillsborough and Pinellas Counties, FL (12-057 and 12-103), 2007-2009.

Table 2-21 Correlations between Pb TSP concentrations from source-oriented and non-source-oriented monitors within Hillsborough and Pinellas Counties, FL (12-057 and 12-103), 2007-2009.

			A	B	C
			Source	Source	Non-source
A	**Source**	R	1.00	-0.09	
		ρ	1.00	-0.08	
		P90	0.00	1.20	0.50
		COD	0.00	0.71	1.00
B	**Source**	R		1.00	
		ρ		1.00	
		P90		0.00	2.20
		COD		0.00	1.00
C	**Non-source**	R			1.00
		ρ			1.00
		P90			0.00
		COD			0.00

Each comparison contains (in order): Pearson rank-order correlation (R), Spearman rank-order correlation (ρ), the difference between the 90th and 10th percentile data (P90), and the coefficient of divergence (*COD*).

Figure 2-46 illustrates Pb monitor locations within Cook County, IL. Eight monitors were located within Cook County, four of which were designated by the Illinois Environmental Protection Agency (IEPA) in data reported to the AQS as source-oriented and the other four were non-source-oriented monitors. Monitor A was situated within 10 km of 6 sources ranging in emissions from 0.14 to 1.08 tons/year (U.S. EPA, 2008a). Monitor A was also sited in the median of Interstate I-90/I-94. Monitor B was located on the northern roadside of Interstate I-290, 5 meters from the closest lane of traffic and was within 10 km of 2 Pb sources (0.41 and 1.08 tons/year) (U.S. EPA, 2008a). Monitor C was also located within 10 km of 6 sources in Cook County and Lake County, IN; the largest of those sources was 2.99 tons/year and was located 8 km southeast of monitor C (U.S. EPA, 2008a). Monitor C was placed on the roof of a high school. Monitor D was located roughly 60 meters west of Interstate I-294 and adjacent to O'Hare International Airport. Monitor E was located on the rooftop of a building rented for government offices in Alsip, IL, a suburb south of Chicago. This location was roughly 1 km north of Interstate I-294 but not located on an arterial road; it was 9 km southeast of a 0.56 tons/year source (U.S. EPA, 2008a). Monitor F was sited in the parking lot of a water pumping station, 100 meters north of Interstate I-90 and 300 meters northwest of the junction between Interstates I-90 and I-94. This site was 2 km north-northwest of a 0.10 tons/year source (U.S. EPA, 2008a). Monitor G was situated atop an elementary

school in a residential neighborhood on the south side of Chicago, roughly 100 meters south of a rail line and over 300 meters west of the closest arterial road. Although not designated as a source monitor, monitor G was located 2 km southwest of facilities emitting 0.30 and 0.41 tons/year (U.S. EPA, 2008a). Monitor H was sited on the grounds of the Northbrook Water Plant. Interstate I-94 curves around this site and was approximately 700 meters from the monitor to the east and around to the north. Figure 2-47 displays seasonal wind roses for Cook County. Wind patterns were quite variable during each season for this area. During the winter, winds mostly came from the west, with smaller contributions from the northwest, southwest, and south. In spring, measurable winds were omni-directional, with the highest winds coming from the south and northeast. Winds originated predominantly from the southwest and south during the summer, with measurable contributions from the northeast as well. In autumn, wind flow was predominantly from the south, but smaller contributions also came from the southwest, west, and northwest.

Figure 2-48 presents seasonal box plots of Pb concentration at the eight monitors located within Cook County. The maximum 95th percentile concentration on this plot was 0.14 $\mu g/m^3$, so the scale of this box plot makes the variability in these data appear wider than the data presented for Los Angeles County and Hillsborough/Pinellas Counties.

Monitor C was in closest proximity to the industrial steel facilities located in Lake County, IN. The average of concentrations measured at monitor C was 0.031 $\mu g/m^3$, with a median of 0.02 $\mu g/m^3$ and a maximum concentration of 0.31 $\mu g/m^3$. In winter, the 95th percentile of data was 0.14 $\mu g/m^3$. The higher values could potentially be attributed to transport of emissions; winds blow from the southeast roughly 10-15% of the time throughout the year. No other monitors in Cook County reported values above the level of the NAAQS.

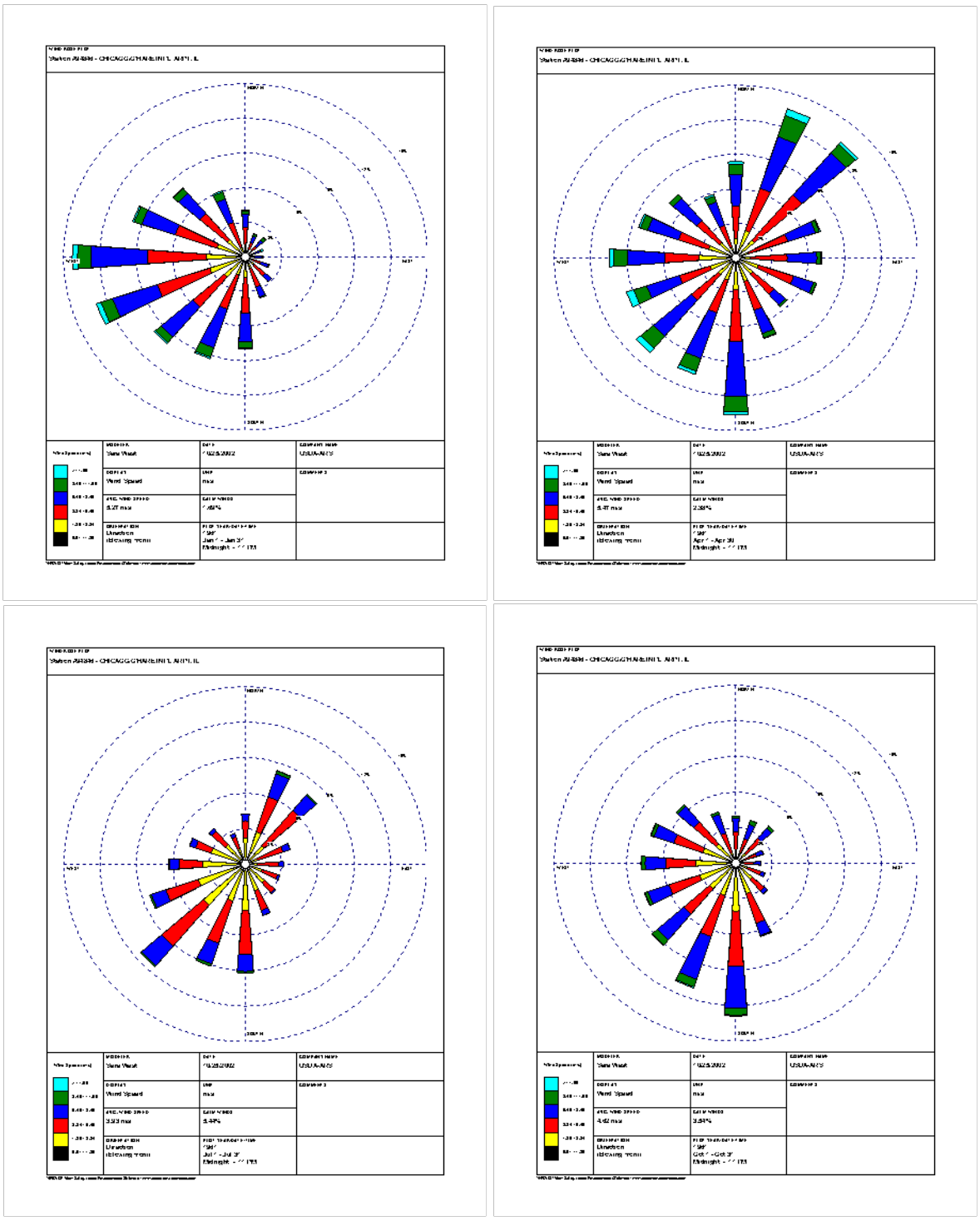
Three “near-road” monitors, A, B, and D can be compared with the other monitors to consider the possibility of roadside resuspension of Pb dust from contemporaneous sources, as discussed in Section 2.2.2.6. It would be expected that resuspension would diminish with distance from the road. The 2 roadside monitors, A and B, reported average concentrations of 0.030 $\mu g/m^3$ and 0.024 $\mu g/m^3$, respectively. The median concentrations for monitors A and B were 0.02 $\mu g/m^3$. Fifteen percent of data were below the MDL for monitor A, and 25% were below the MDL for monitor B. Note that data obtained from monitor A may reflect industrial emissions as well. Monitor D was located roughly 60 meters from the closest interstate and 570 meters from the closest runway at O’Hare International Airport. The average concentration at this site was 0.012 $\mu g/m^3$, and 85% of data were below the MDL. Non-source monitors, E, F, G, and H had average concentrations of 0.011-0.017 $\mu g/m^3$. It is possible that the difference between Pb

concentrations at monitors A and B and Pb concentrations at the other monitors was related to proximity to the roadway, although this cannot be stated with certainty without source apportionment data to confirm or refute the influence of industrial plumes from Lake County, IN or local sources at each of the monitors.

Comparison among the monitor data demonstrates a high degree of spatial variability (Table 2-22). None of the source-oriented monitors were well correlated with each other. The highest correlation between source-oriented monitors occurred for monitors (A and B [R = 0.32, ρ = 0.26]). This might have reflected more substantial differences related to the additional influence of industrial sources nearby monitor A. Monitors (C and D) were uncorrelated with each other and with monitors (A and B), likely because their exposure to sources was substantially different. The source-oriented and non-source-oriented monitors were generally not well correlated. The highest Spearman correlation occurred between monitors D and H (ρ = 0.53), but Pearson correlation was much lower for this pair (R = 0.19). Both were located on the north side of Cook County, but monitor H was roughly 20 km northeast of monitor D. Winds blew from the southwest roughly 20-30% of the time throughout the year and from the northeast 20-25% of the time between the months of March and July, so the correlation may have been related to a common signal transported across both sites. Monitors B and F (R = 0.52, ρ = 0.46) were also moderately correlated. Monitor F is roughly 12 km northeast of monitor B, so the same common wind influence for monitors D and H may have also caused the moderate correlation between monitors (B and F). Monitor F was also moderately correlated with the other 3 non-source monitors (R = 0.42 to 0.54, ρ = 0.36 to 0.45), and the correlation between monitors (E and G) was moderate (R = 0.65, ρ = 0.40). The data from monitor H did not correlate well with those from monitors E and G. The non-source monitors were oriented from north to south over a distance of roughly 50 km in the following order: monitor H, monitor F, monitor G, and monitor E. The correlation pattern may have been related to distance between samplers. Monitor H was located in the suburb of Northbrook, monitors F and G were sited within the Chicago city limits, and monitor E was situated in a town near the south side of Chicago. Differences among land use may have been related to the lack of correlation of the monitor H data with those from monitors E and G. It is likely that data from monitor F was at times better correlated with monitors E and G and at other times with monitor H, since it had moderate correlation with all three other non-source monitors.

Top: view of all Pb FRM monitors in Cook County.
Bottom left: Close up of the high traffic site around monitor A.
Bottom right: Close up of O'Hare International Airport adjacent to monitor D.

Figure 2-46 Pb TSP Monitor locations within Cook County, IL (17-031), 2007-2009.



Note: Clockwise from the top left: January, April, July, and October. Note that the wind percentages vary from month to month.
Source: NRCS (2011)

Figure 2-47 Wind roses for Cook County, IL, obtained from meteorological data at O'Hare International Airport, 1961-1990.

Site	A	B	C	D	E	F	G	H
SITE ID	17-031-0026	17-031-6003	17-031-0022	17-031-3103	17-031-0001	17-031-0052	17-031-3301	17-031-4201
MEAN	0.030	0.024	0.031	0.012	0.013	0.017	0.017	0.011
SD	0.020	0.013	0.036	0.0062	0.0078	0.0098	0.0097	0.0031
OBS	179	175	177	168	177	175	171	168
% BELOW MDL	15	25	25	85	75	55	50	95
Source orientation	**Source**	**Source**	**Source**	**Source**	**Non-source**	**Non-source**	**Non-source**	**Non-source**

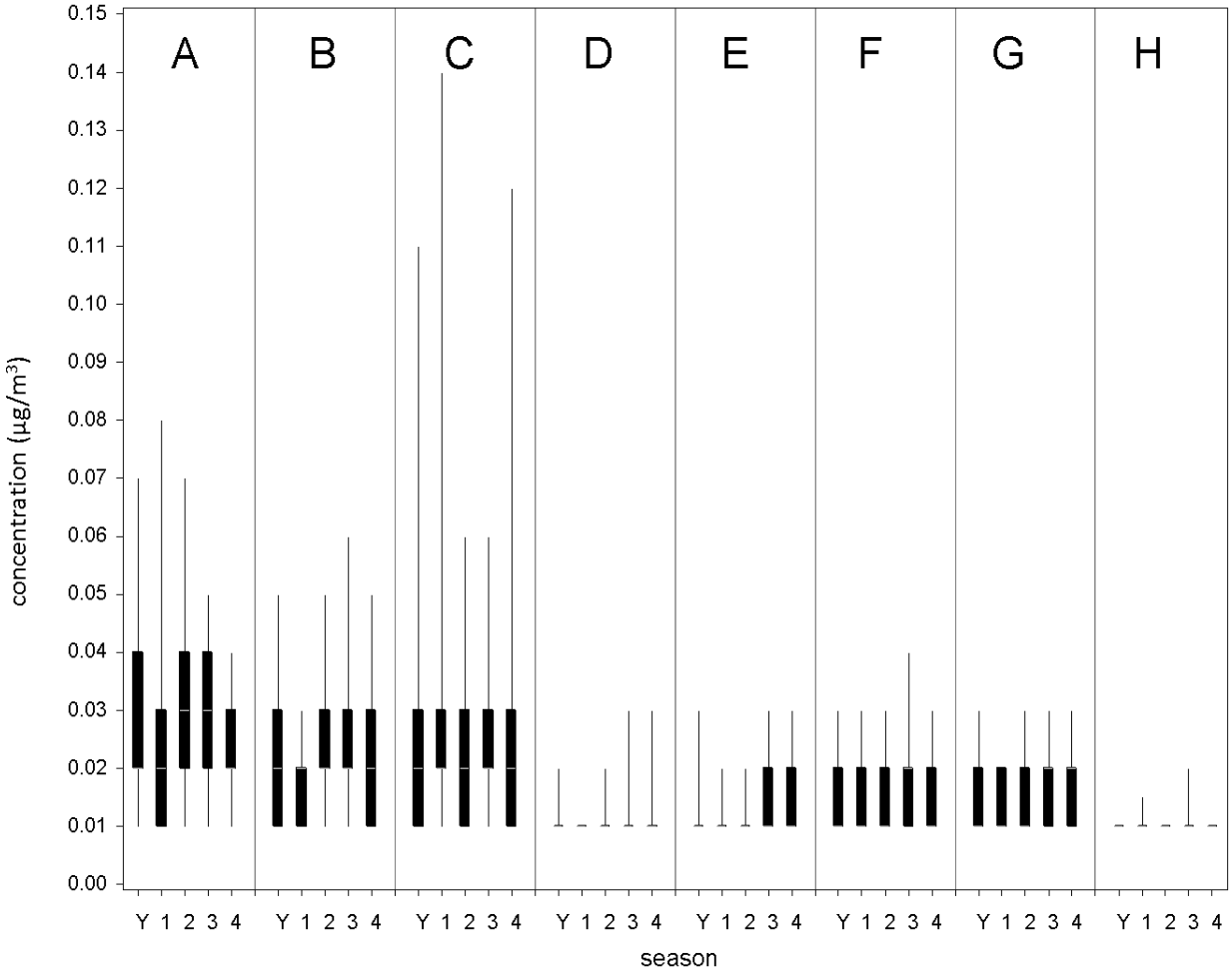


Figure 2-48 Box plots of annual and seasonal 24-h Pb TSP concentrations (µg/m^3) from source-oriented and non-source-oriented monitors within Cook County, IL (17-031), 2007-2009.

Table 2-22 Correlations between Pb TSP concentrations from source-oriented and non-source-oriented monitors within Cook County, IL (17-031), 2007-2009.

			A	B	C	D	E	F	G	H
			Source	**Source**	**Source**	**Source**	**Non-Source**	**Non-Source**	**Non-Source**	**Non-Source**
A	**Source**	R	1.00	0.32	0.00	0.05	0.17	0.39	0.34	0.06
		ρ	1.00	0.26	-0.01	0.08	0.06	0.32	0.18	0.06
		P90	0.00	0.03	0.06	0.04	0.04	0.03	0.03	0.04
		COD	0.00	0.29	0.38	0.43	0.41	0.36	0.36	0.45
B	**Source**	R		1.00	0.14	0.07	0.54	0.52	0.60	0.06
		ρ		1.00	0.05	0.10	0.32	0.46	0.35	-0.01
		P90		0.00	0.04	0.03	0.03	0.02	0.02	0.03
		COD		0.00	0.33	0.36	0.34	0.29	0.30	0.40
C	**Source**	R			1.00	0.01	0.24	0.05	0.19	-0.04
		ρ			1.00	0.04	0.16	0.10	0.17	0.06
		P90			0.00	0.05	0.05	0.04	0.05	0.05
		COD			0.00	0.40	0.39	0.35	0.35	0.42
D	**Source**	R				1.00	0.18	0.12	0.08	0.19
		ρ				1.00	0.21	0.37	0.07	0.53
		P90				0.00	0.01	0.01	0.02	0.01
		COD				0.00	0.19	0.24	0.28	0.15
E	**Non-Source**	R					1.00	0.42	0.65	-0.01
		ρ					1.00	0.36	0.40	0.07
		P90					0.00	0.02	0.01	0.01
		COD					0.00	0.24	0.24	0.20
F	**Non-Source**	R						1.00	0.54	0.42
		ρ						1.00	0.41	0.45
		P90						0.00	0.01	0.02
		COD						0.00	0.24	0.26
G	**Non-Source**	R							1.00	0.01
		ρ							1.00	0.05
		P90							0.00	0.02
		COD							0.00	0.27
H	**Non-Source**	R								1.00
		ρ								1.00
		P90								0.00
		COD								0.00

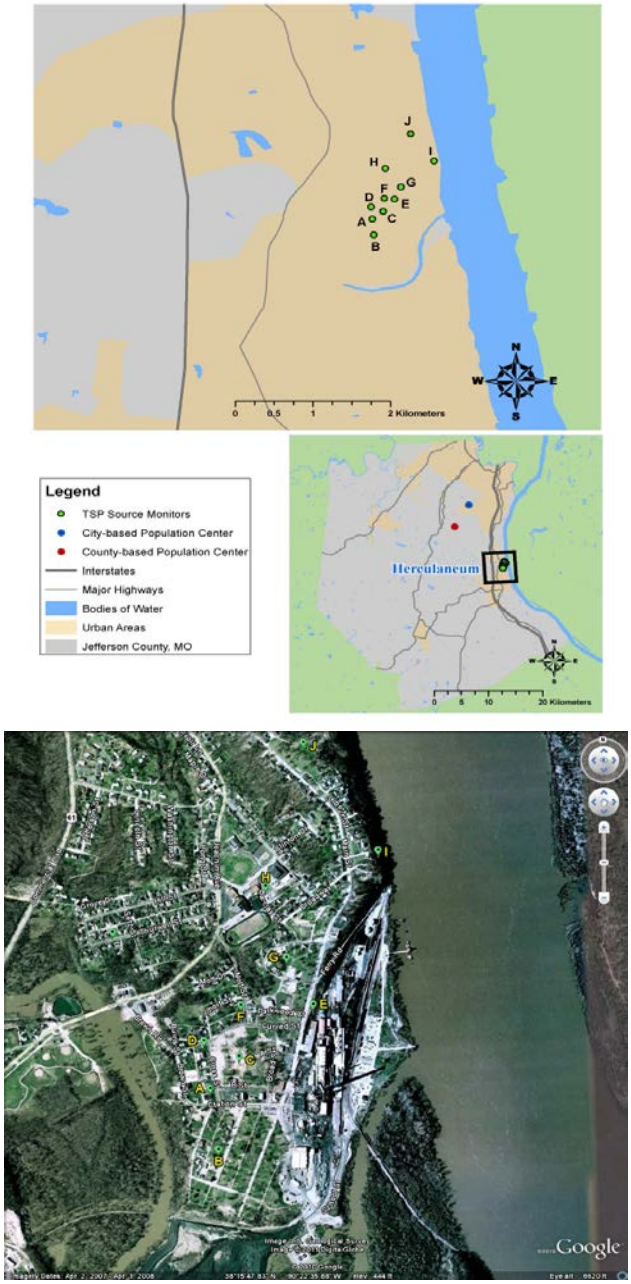
Each comparison contains (in order): Pearson rank-order correlation (R), Spearman rank-order correlation (p), the difference between the 90th and 10th percentile data (P90), and the coefficient of divergence (*COD*).

Figure 2-49 illustrates Pb monitor locations with Jefferson County, MO. Ten source-oriented monitors surrounded the Doe Run primary Pb smelter in Herculaneum, MO on the west and northwestern sides. The largest distance between these monitors was approximately 1.5 km. Monitor E located on the Doe Run facility roughly 20 meters west of the nearest building. Monitors A, B, C, D, F, G, and H were all located approximately 200 meters west of the facility. Monitors D, E, and H were situated alongside service roads to the facility. Monitor I was sited 100 meters north of the smelter, and monitor J was located approximately 600 meters northwest of the facility. The Doe Run smelter was the only active primary smelter in the U.S. at the time of this review, and the facility was estimated to have emitted 41.1 tons Pb/year (U.S. EPA, 2008f). Figure 2-50 displays seasonal wind roses for Jefferson County. During winter, predominant winds originated from the northwest, with a smaller fraction of calmer winds originating in the south-southeast. During the spring, the south-southeasterly winds became more prevalent with a measurable fraction of stronger winds still originating in the north-northwest. In the summer, winds were omni-directional and generally calmer. A slightly larger percentage came from the south compared with other wind directions. Autumn winds were most predominantly south-southeastern, with a smaller fraction from the west and northwest.

Figure 2-51 illustrates the seasonal distribution of concentrations at monitors A-J in Jefferson County. The annual average concentrations ranged from 0.18 to 1.36 $\mu g/m^3$ across the monitors. The maximum concentration was measured at monitor C to be 21.6 $\mu g/m^3$, which was 144 times higher than the level of the standard. For this monitor, the 25th percentile of the data was at the level of the standard. In general, median and 75th percentile concentrations were highest during the springtime and second highest during the fall. These seasons coincide with periods when the southeastern winds were stronger and more prevalent. Because the Doe Run facility had two 30-meter stacks (Bennett, 2007), it is possible that the Pb measured at the closer monitors were due to either fugitive emissions from the plant; or, if vehicles and ground equipment were operated nearby, the previously-deposited emissions from the plant were resuspended.

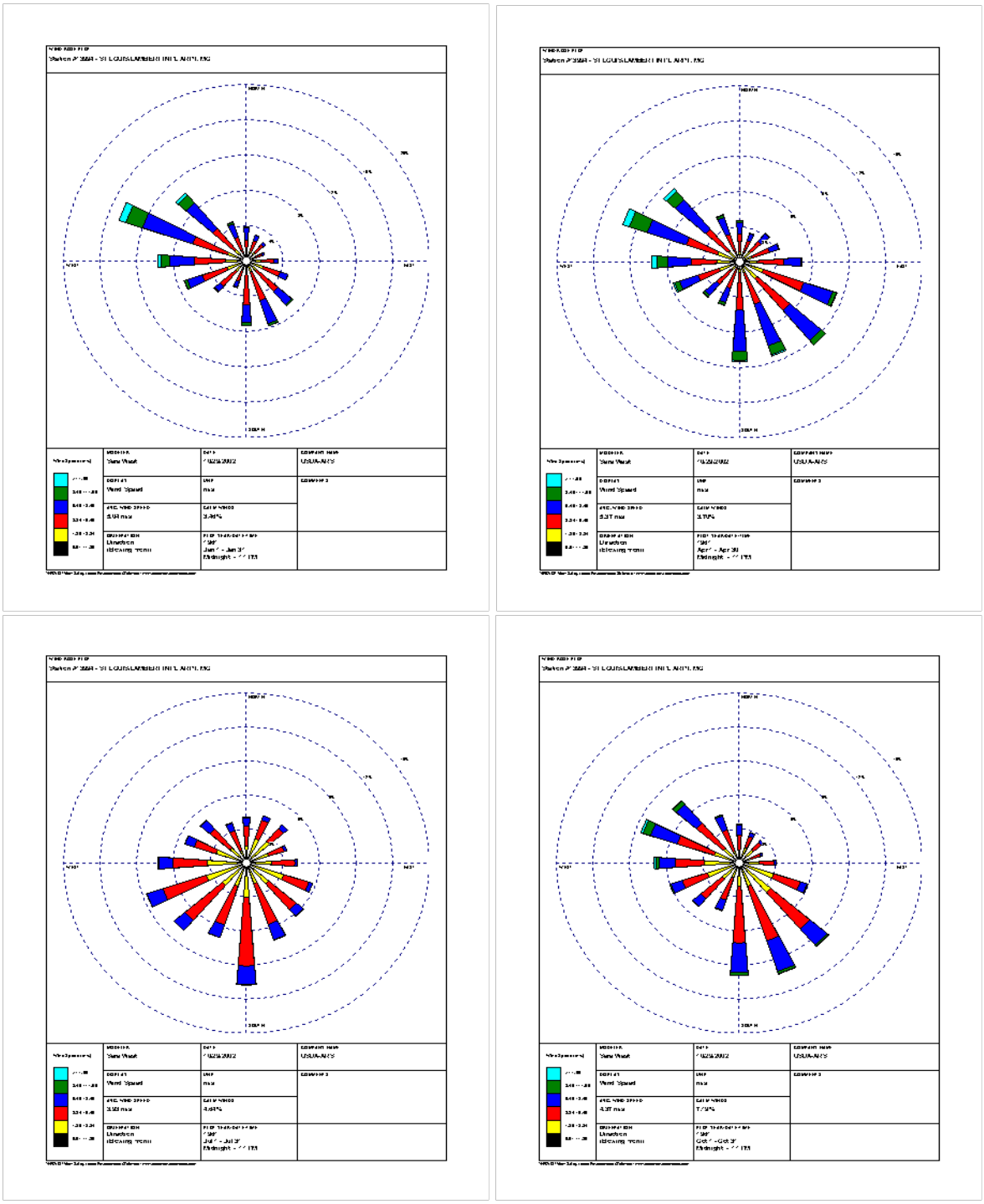
Spatial variability among the monitors is lower than at many sites, because the monitors are relatively close together and are located on one side of the same source (Table 2-23). Correlations range substantially ($R = -0.03$ to 0.96, $\rho = -0.04$ to 0.96). High correlations ($R \geq 0.75$, $\rho \geq 0.75$) occurred for monitors (A and C), (A and D), (C and D), (D and F), (E and F), (G and H), and (I and J). Monitors (A and C), (A and D), (C and D), (D and F), (E and F), and (G and H) are all within 250 meters of each other. For the highest correlation ($R = 0.96$, $\rho = 0.96$, [for monitors E and F]), monitor F is 250 meters directly east of monitor E. Low correlation ($R \leq 0.25$, $\rho \leq 0.25$) generally occurred when monitors B, I, and J were compared with monitors A, C, D, E, F, G, and H. Monitors B, I, and J were on the outskirts of the measurement area and so were likely oriented such that the

southeasterly winds did not carry pollutants to these sites concurrently with the signal recorded by the other monitors.



Note: All monitors surround the Doe Run industrial facility. Top: Map view of all monitors in Jefferson County. Bottom: Satellite view of the monitors and the Doe Run facility.

Figure 2-49 Pb TSP Monitor locations within Jefferson County, MO (29-099), 2007-2009.



Note: Clockwise from top left: January, April, July, and October. Note wind percentages vary from month to month.
Source: NRCS (2011)

Figure 2-50 Wind roses for Jefferson County, MO, obtained from meteorological data at St. Louis/Lambert International Airport, 1961-1990.

Site	A	B	C	D	E	F	G	H	I	J
SITE ID	29-099-0022	29-099-0024	29-099-0015	29-099-0023	29-099-0004	29-099-0020	29-099-0021	29-099-0005	29-099-0011	29-099-0013
MEAN	0.43	0.36	1.36	0.39	1.12	0.69	0.75	0.29	0.34	0.18
SD	0.54	0.49	1.97	0.54	1.67	1.01	1.25	0.59	0.85	0.33
OBS	622	209	1E3	632	1E3	575	953	351	366	177
% BELOW MDL	0	5	0	0	5	0	5	25	5	15
Source orientation	Source	Source	Source	Source	Source	Source	Source	Source	Source	Source

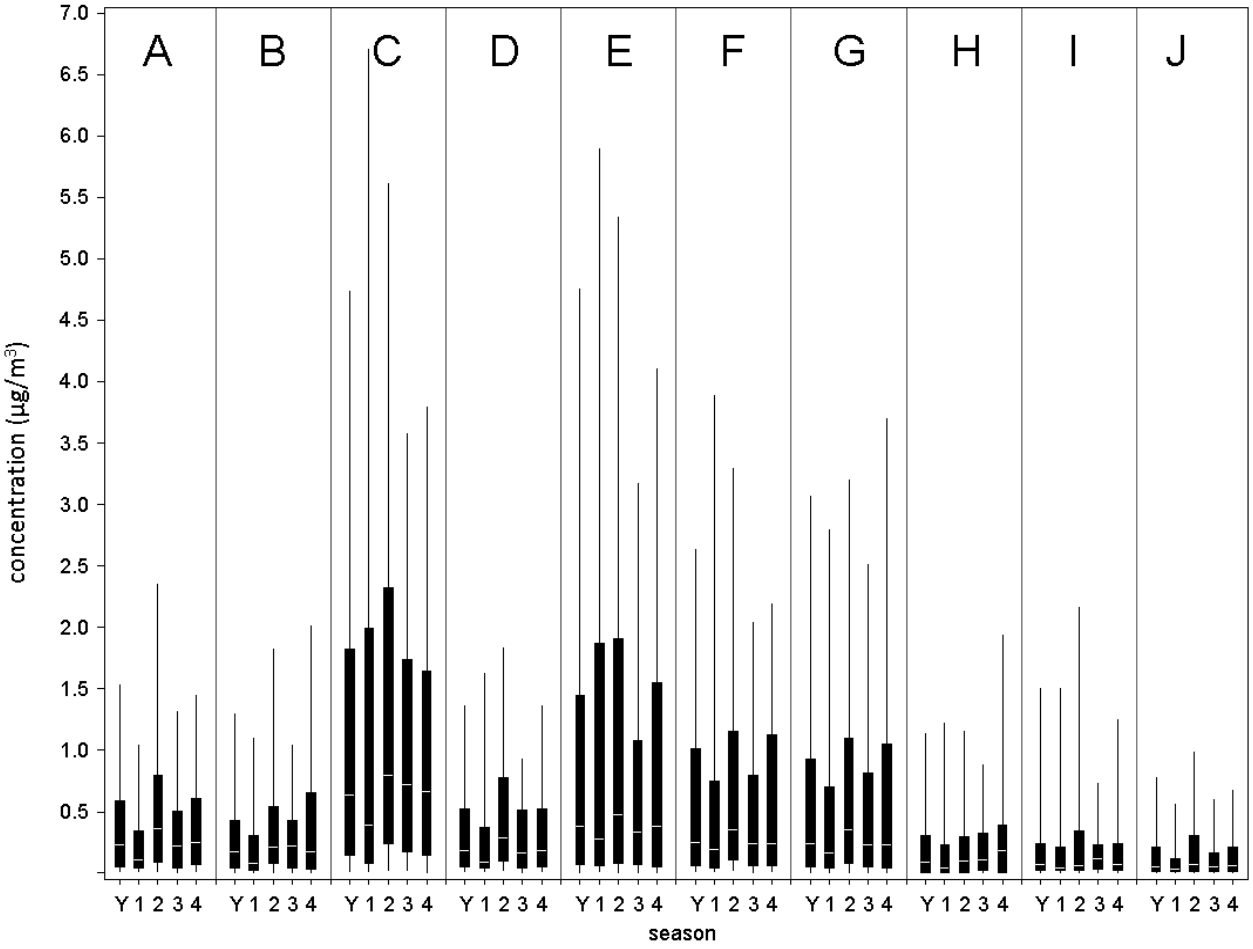


Figure 2-51 Box plots of annual and seasonal 24-h Pb TSP concentrations (µg/m^3) from source-oriented and non-source-oriented monitors within Jefferson County, MO (29-099), 2007-2009.

Table 2-23 Correlations between Pb TSP concentrations from source-oriented and non-source-oriented monitors within Jefferson County, MO (29-099), 2007-2009.

			A	B	C	D	E	F	G	H	I	J
			Source	**Source**	**Source**	**Source**	**Source**	**Source**	**Source**	**Source**	**Source**	**Source**
A	**Source**	R	1.00	0.66	0.80	0.84	0.60	0.65	0.33	0.32	0.07	0.05
		ρ	1.00	0.59	0.80	0.83	0.57	0.64	0.33	0.35	0.07	0.05
		P90	0.00	0.71	1.55	0.42	1.93	1.14	1.41	0.74	0.92	0.78
		COD	0.00	0.46	0.48	0.30	0.55	0.45	0.57	0.64	0.67	0.69
B	**Source**	R		1.00	0.54	0.40	0.15	0.15	0.08	0.16	0.11	0.01
		ρ		1.00	0.53	0.43	0.10	0.14	0.07	0.22	0.10	0.09
		P90		0.00	1.86	0.87	2.77	1.96	2.08	0.94	1.04	0.91
		COD		0.00	0.58	0.51	0.69	0.62	0.68	0.68	0.65	0.65
C	**Source**	R			1.00	0.86	0.56	0.72	0.28	0.32	-0.03	-0.03
		ρ			1.00	0.86	0.59	0.72	0.26	0.27	-0.04	0.04
		P90			0.00	1.56	2.26	1.26	2.94	2.65	3.18	2.60
		COD			0.00	0.50	0.50	0.46	0.60	0.74	0.73	0.73
D	**Source**	R				1.00	0.70	0.80	0.41	0.48	0.17	0.10
		ρ				1.00	0.71	0.80	0.41	0.56	0.14	0.18
		P90				0.00	1.83	1.02	1.38	0.76	0.88	0.70
		COD				0.00	0.50	0.36	0.53	0.61	0.63	0.66
E	**Source**	R					1.00	0.96	0.57	0.53	0.09	0.14
		ρ					1.00	0.96	0.54	0.46	0.06	0.16
		P90					0.00	0.86	2.16	2.50	3.09	2.57
		COD					0.00	0.35	0.49	0.66	0.70	0.72
F	**Source**	R						1.00	0.56	0.56	0.12	0.20
		ρ						1.00	0.56	0.54	0.10	0.19
		P90						0.00	1.13	1.51	1.74	1.40
		COD						0.00	0.47	0.63	0.65	0.70
G	**Source**	R							1.00	0.85	0.36	0.34
		ρ							1.00	0.87	0.28	0.38
		P90							0.00	1.53	2.10	2.08
		COD							0.00	0.61	0.63	0.66

Table 2-23 (Continued): Correlations between Pb TSP concentrations from source-oriented and non-source-oriented monitors within Jefferson County, MO (29-099), 2007-2009.

			A	B	C	D	E	F	G	H	I	J
H	**Source**	R								1.00	0.24	0.33
		ρ								1.00	0.20	0.30
		P90								0.00	0.89	0.56
		COD								0.00	0.67	0.65
I	**Source**	R									1.00	0.87
		ρ									1.00	0.79
		P90									0.00	0.62
		COD									0.00	0.48
J	**Source**	R										1.00
		ρ										1.00
		P90										0.00
		COD										0.00

Each comparison contains (in order): Pearson rank-order correlation (R), Spearman rank-order correlation (ρ), the difference between the 90th and 10th percentile data (P90), and the coefficient of divergence (*COD*).

Figure 2-52 illustrates Pb monitor locations in Cuyahoga County, OH. Five monitors are located within Cuyahoga County, three of which were designated by the Ohio EPA (OEPA) as source-oriented and the other two were non-source-oriented monitors. Monitors A, B, and C were all located within 1-10 km of six 0.1 tons/year source facilities and one 0.2 tons/year source (U.S. EPA, 2008g). Additionally, monitor B was located 30 meters north of the Ferro Corporation headquarters. This facility was stated in the 2005 NEI to have no emissions, but it was thought by the OEPA to be the source of exceedances at this monitor (U.S. EPA, 2008g). Monitor A was sited roughly 300 meters south of the Ferro Corporation facility. Monitor C was located 2.2 km west-northwest of the 0.5 tons/year Victory White Metal Co. facility. Monitor C was also roughly 250 meters southeast of Interstate I-490. Monitors D and E were designated as non-source-oriented monitors, although monitor D was just 600 meters further from the Victory White Metal facility than was monitor C. Monitor D was sited on a residential street located 50 meters north of Interstate I-490. Monitor E was located on the rooftop of a building within 20 meters of a four-lane arterial road. Figure 2-53 displays seasonal wind roses for Cuyahoga County. During winter, summer, and autumn, the predominant winds were from the southwest, with stronger winds recorded during the winter. In the spring, the strongest winds still emanated from the south-southwest, but measurable winds were also scattered from the northeast to the northwest.

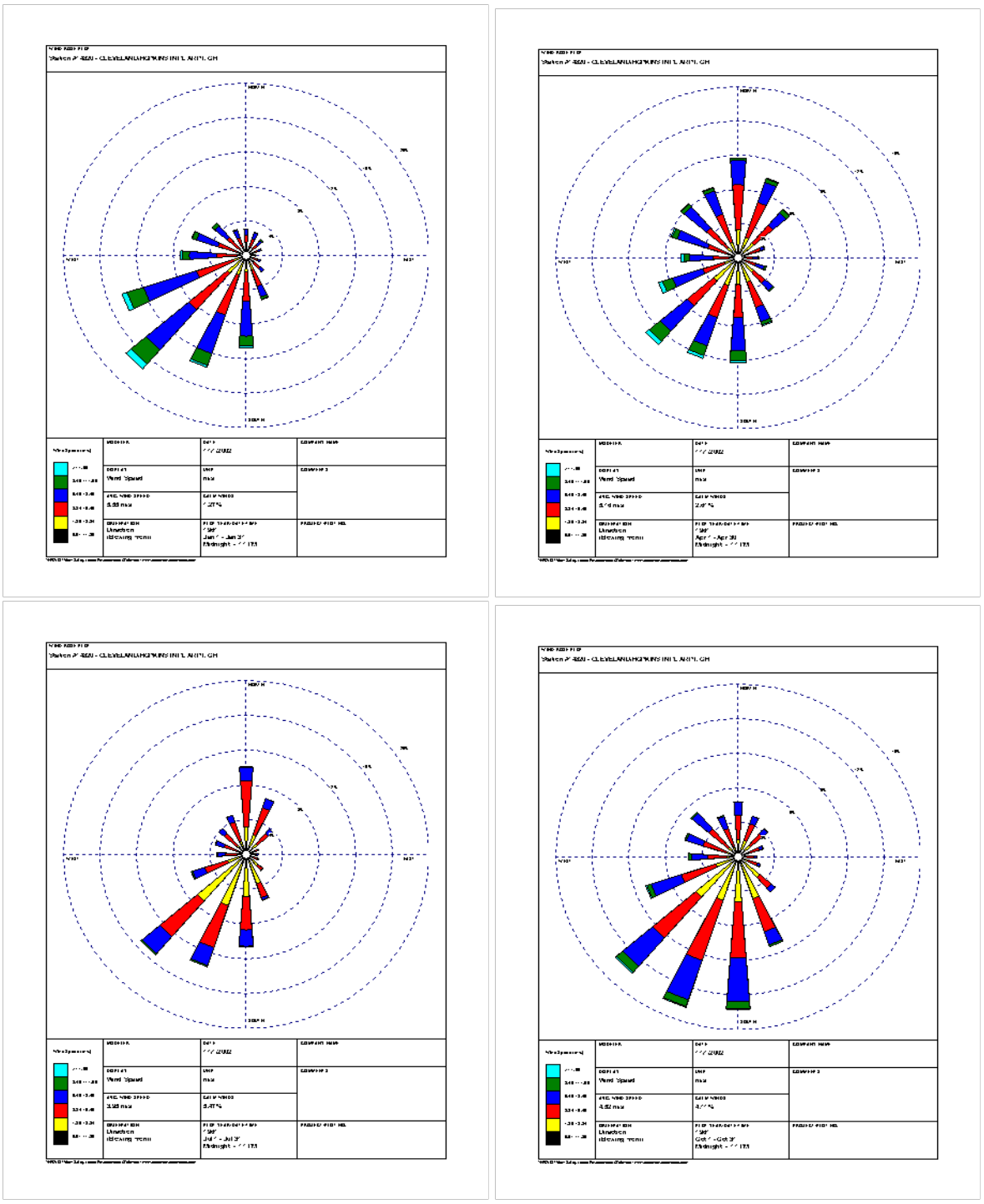
Figure 2-54 illustrates the seasonal distribution of Pb concentration data at the five monitoring sites. The influence of southern winds, along with close proximity to a

potentially-emitting facility, could have caused the elevated concentrations observed at monitor B (average: 0.10 μg/m^3). The 80th percentile of data was at the level of the NAAQS at this monitor, and during autumn the 60th percentile of data met the level of the NAAQS. The maximum concentration during fall and for the monitor year-round was 0.22 μg/m^3. Concentration data from all other monitors were below the level of the NAAQS. For monitor A, the average concentration was 0.025 μg/m^3, and the median reached 0.04 μg/m^3 during the summer. Maximum concentration at this monitor was 0.07 μg/m^3. Concentrations at monitor C averaged 0.017 μg/m^3, and those at monitors D and E averaged 0.014 μg/m^3 and 0.013 μg/m^3, respectively. Maximum concentrations reached 0.04 μg/m^3 at all three monitors.

The level of spatial variability is illustrated by the intersampler correlations presented in Table 2-24. Monitors A and B appear to be anticorrelated ($R = -0.06$, $\rho = -0.13$). If the Ferro site was the dominant source in this area, then the anticorrelation was likely caused by the positioning of monitors A and B on opposite sides of that facility. At any given time, potential emissions from the Ferro plant may have affected monitors A and B at distinct times. Monitors C, D, and E correlated moderately to well with each other ($R = 0.37$ to 0.74, $\rho = 0.67$ to 0.77). Given that all 3 monitors are separated by roughly 2.8 km, it is possible that the relatively high correlations related to common sources, as suggested in the previous paragraph. Little correlation was observed between the source-oriented and non-source-oriented monitors.

Note: Top: view of all Pb FRM monitors in Cuyahoga County. Bottom left: Close up of industrial site around monitors A and B. Bottom right: Close up of monitor D north of Interstate I-490.

Figure 2-52 Pb TSP Monitor locations within Cuyahoga County, OH (39-035), 2007-2009.



Note: Clockwise from top left: January, April, July, and October. Note wind percentages vary from month to month.
Source: NRCS (2011)

Figure 2-53 Wind roses for Cuyahoga County, OH, obtained from meteorological data at Cleveland/Hopkins International Airport, 1961-1990.

Site	A	B	C	D	E
SITE ID	39-035-0050	39-035-0049	39-035-0061	39-035-0038	39-035-0042
MEAN	0.025	0.10	0.017	0.014	0.013
SD	0.018	0.060	0.010	0.0072	0.0076
OBS	36	36	36	35	36
% BELOW MDL	20	0	30	45	45
Source orientation	Source	Source	Source	Non-source	Non-source

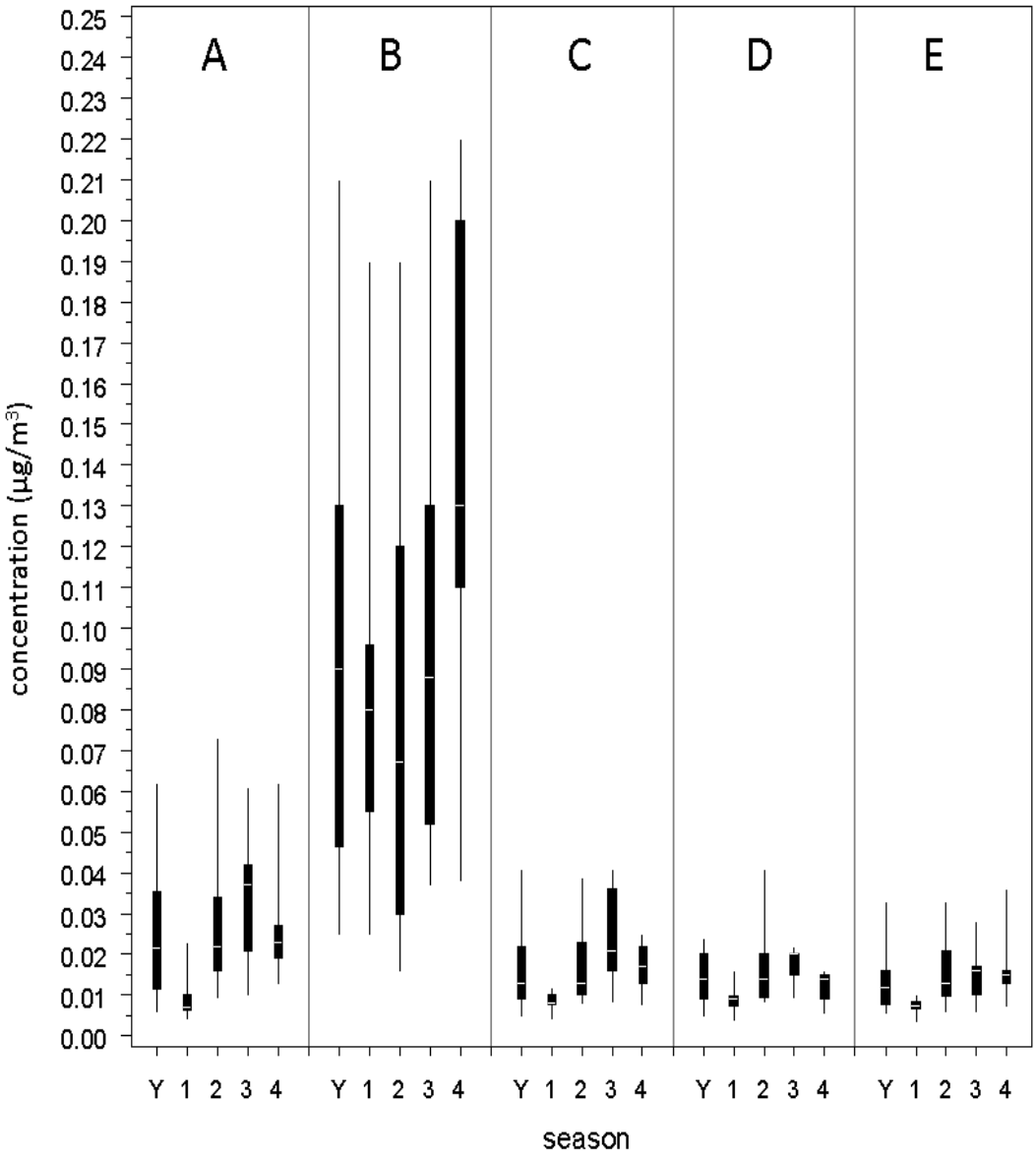


Figure 2-54 Box plots of annual and seasonal 24-h Pb TSP concentrations (µg/m^3) from source-oriented and non-source-oriented monitors within Cuyahoga County, OH (39-035), 2007-2009.

Table 2-24 Correlations between Pb TSP concentrations from source-oriented and non-source-oriented monitors within Cuyahoga County, OH (39-035), 2007-2009.

			A	B	C	D	E
			Source	Source	Source	Non-Source	Non-Source
A	**Source**	R	1.00	-0.06	0.21	0.17	0.24
		ρ	1.00	-0.13	0.24	0.19	0.21
		P90	0.00	0.18	0.05	0.04	0.05
		COD	0.00	0.64	0.33	0.35	0.37
B	**Source**	R		1.00	0.26	0.43	0.11
		ρ		1.00	0.31	0.24	0.34
		P90		0.00	0.18	0.19	0.19
		COD		0.00	0.69	0.71	0.73
C	**Source**	R			1.00	0.74	0.51
		ρ			1.00	0.77	0.67
		P90			0.00	0.01	0.01
		COD			0.00	0.17	0.18
D	**Non-Source**	R				1.00	0.37
		ρ				1.00	0.67
		P90				0.00	0.01
		COD				0.00	0.17
E	**Non-Source**	R					1.00
		ρ					1.00
		P90					0.00
		COD					0.00

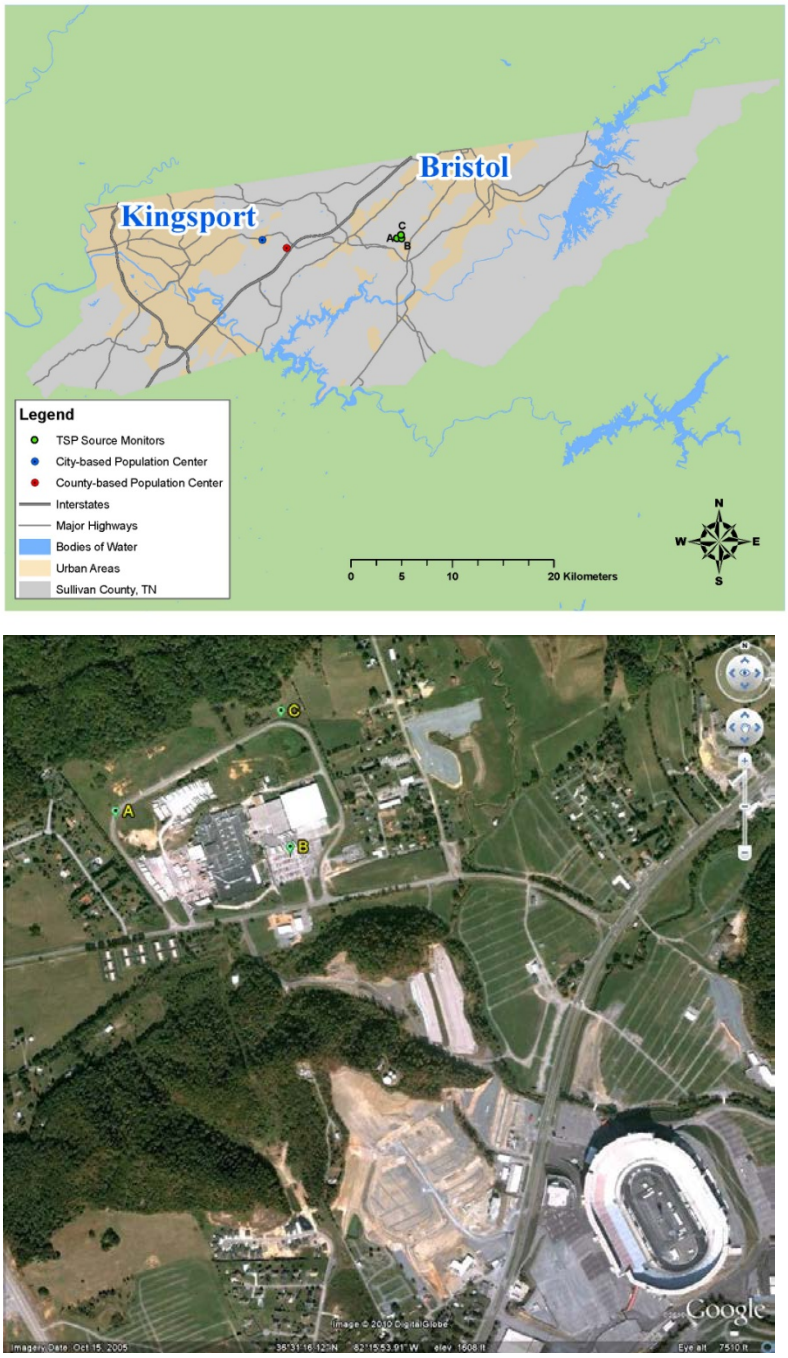
Each comparison contains (in order): Pearson rank-order correlation (R), Spearman rank-order correlation (p), the difference between the 90th and 10th percentile data (P90), and the coefficient of divergence (*COD*).

Figure 2-55 illustrates Pb monitor locations within Sullivan County, TN. Three source-oriented monitors were situated around an Exide Pb recycling facility emitting 0.78 tons/year (U.S. EPA, 2008h). Monitors A and C are positioned along the facility's service road and are approximately 100 meters and 200 meters away from the facility, respectively. Monitor A is directly next to the road, and monitor C is roughly 15 meters from the road. Monitor B is located in the facility's parking lot roughly 50 meters from the closest building. The facility and all three monitors are approximately 1.5 km northwest of the Bristol Motor Speedway and Dragway racetracks, which hosts a variety of auto races each year, including NASCAR, KART, and drag racing. Although the NASCAR circuit no longer uses tetraethyl Pb as an anti-knock agent in its fuel, some of

the smaller racing circuits continue to do so. However, the speedway is rarely upwind of the monitoring sites and so likely had minimal influence on the reported concentrations. Figure 2-56 displays seasonal wind roses for Sullivan County. During winter and spring, the predominant winds come from the southwest and west. In the summer, the percentage of wind coming from the west and southwest is roughly equal to that for wind coming from the east and northeast, although the easterly winds are calmer. During autumn, winds come predominantly from the northeast and east, although these winds tend to be calmer than those originating from the southwest and west.

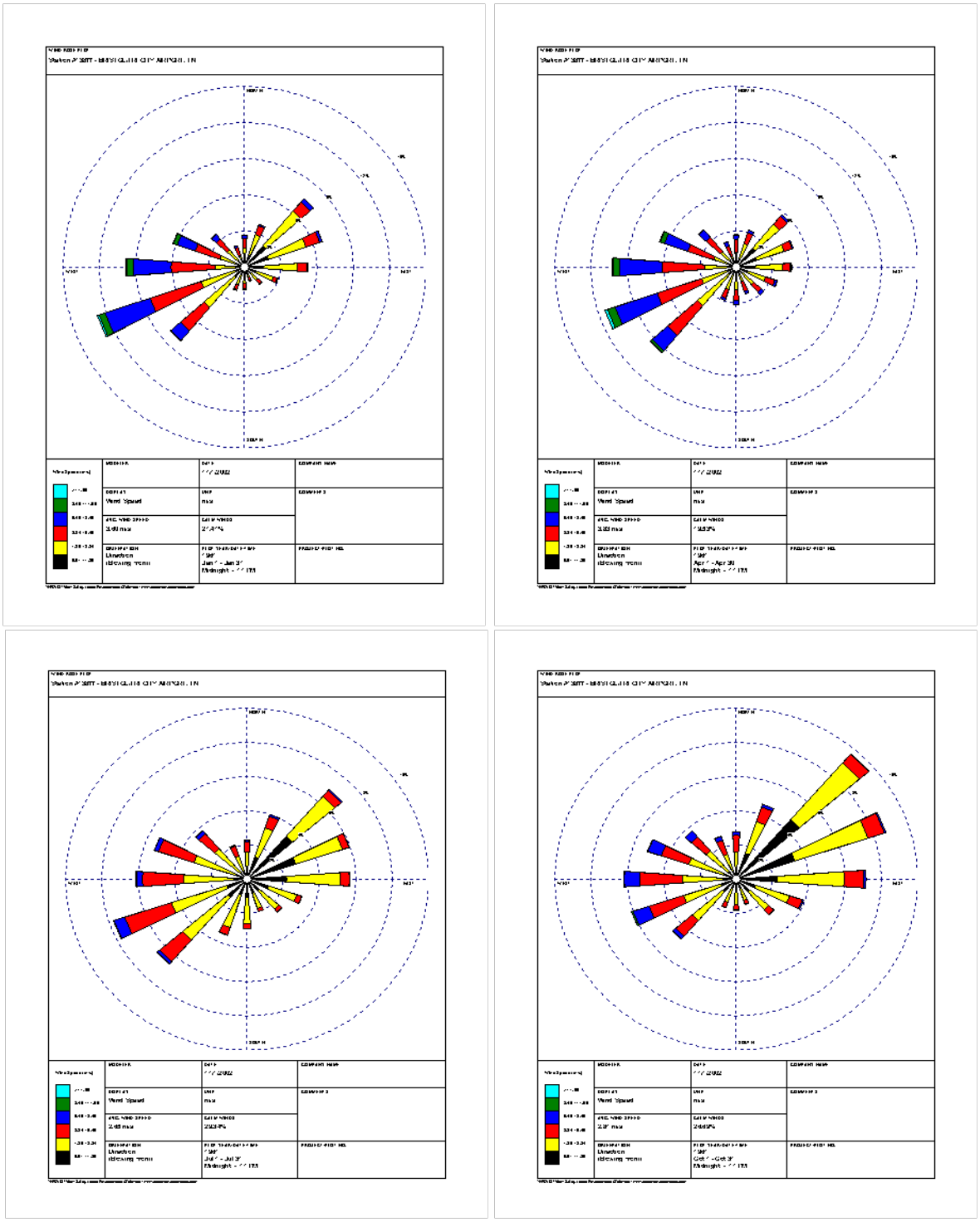
The data presented in Figure 2-57 illustrates that concentrations above the level of the NAAQS occurred frequently at the monitors. The average concentrations at monitors A, B, and C were 0.11 $\mu g/m^3$, 0.051 $\mu g/m^3$, and 0.059 $\mu g/m^3$, respectively. Median concentrations were 0.08 $\mu g/m^3$, 0.03 $\mu g/m^3$, and 0.04 $\mu g/m^3$, respectively. The 75th percentile of year-round data at monitor A was at the level of the NAAQS, while the 95th percentile of data were below the NAAQS level for monitors B and C. The maxima at each monitor were 0.76 $\mu g/m^3$, 0.26 $\mu g/m^3$, and 0.43 $\mu g/m^3$ for monitors A, B, and C. The concentrations measured at monitor A tended to be higher because the predominant and stronger winds came from the southwest, so in many cases monitor A was upwind of the facility. It is possible that Pb that had either deposited or was stored in waste piles became readily resuspended by traffic-related turbulence and was measured at monitor A since that monitor was closest to the road. The slightly higher concentrations at monitor A compared with those from monitor C are consistent with the southwestern winds.

Not surprisingly, the correlations of monitor A with monitors B and C ($R = 0.06$ to 0.14, $\rho = -0.04$ to 0.13) were quite low (Table 2-25). The correlation between monitors B and C was moderate ($R = 0.31$, $\rho = 0.45$). It makes sense that the correlation for these monitors would be somewhat higher because they are both oriented to the east of the Pb recycling facility, although monitor C is to the northeast and monitor B to the east-southeast.



Note: Top: Map, bottom: Satellite image. Monitors A, B, and C surround the Exide Pb recycling facility. Just to the southeast is the Bristol motor speedway.

Figure 2-55 Pb TSP Monitor locations within Sullivan County, TN (47-163), 2007-2009.



Source: NRCS (2011)

Note: Clockwise from top left: January, April, July, and October. Note that the wind percentages vary from month to month.

Figure 2-56 Wind roses for Sullivan County, TN, obtained from meteorological data at Bristol/Tri City Airport, 1961-1990.

Site	A	B	C
SITE ID	47-163-3001	47-163-3002	47-163-3003
MEAN	0.11	0.051	0.059
SD	0.11	0.036	0.047
OBS	334	362	345
% BELOW MDL	0	0	0
Source orientation	**Source**	**Source**	**Source**

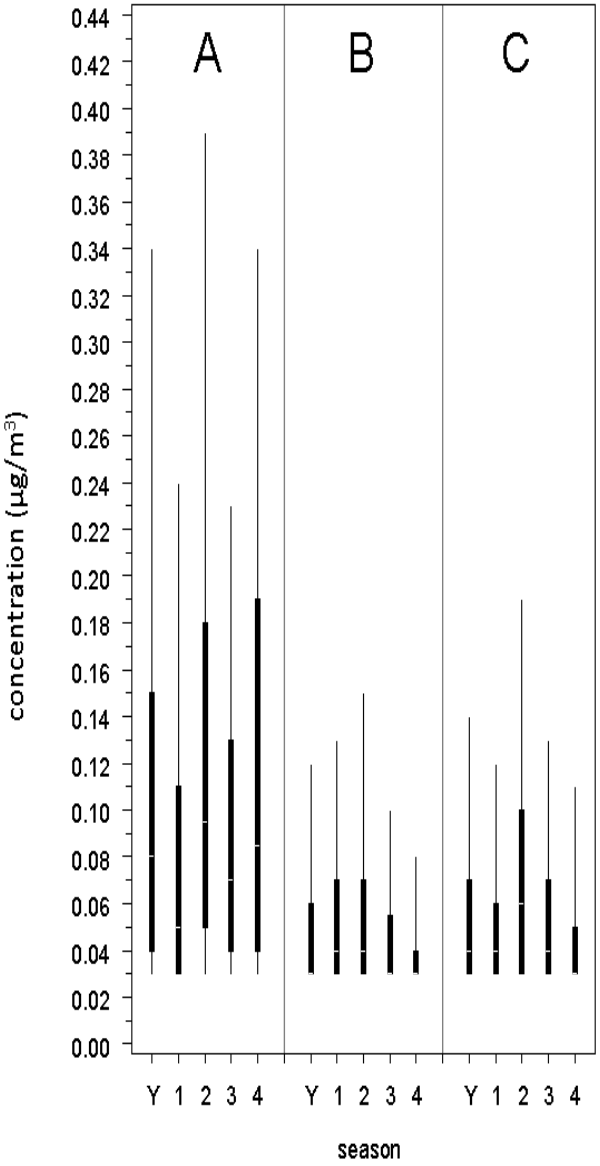


Figure 2-57 Box plots of annual and seasonal 24-h Pb TSP concentrations (µg/m^3) from source-oriented monitors within Sullivan County, TN (47-163), 2007-2009.

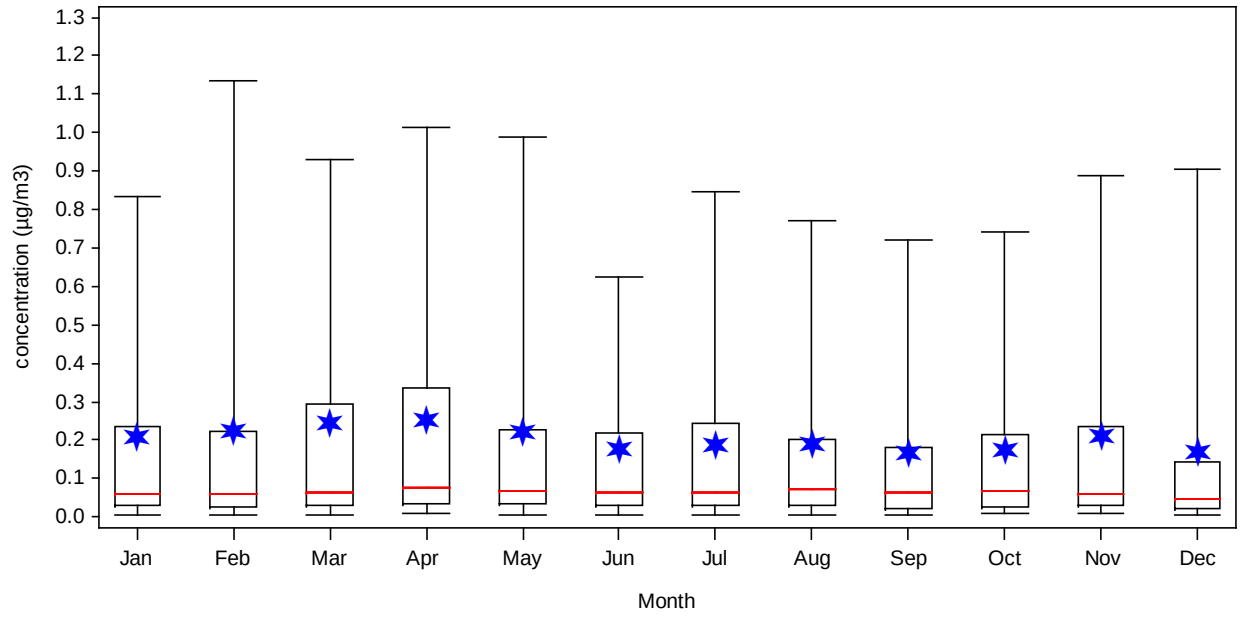
Table 2-25 Correlations between Pb TSP concentrations from source-oriented monitors within Sullivan County, TN (47-163), 2007-2009.

			A	B	C
			Source	**Source**	**Source**
A	**Source**	R	1.00	0.06	0.14
		ρ	1.00	-0.04	0.13
		P90	0.00	0.21	0.19
		COD	0.00	0.47	0.43
B	**Source**	R		1.00	0.31
		ρ		1.00	0.45
		P90		0.00	0.06
		COD		0.00	0.23
C	**Source**	R			1.00
		ρ			1.00
		P90			0.00
		COD			0.00

Each comparison contains (in order): Pearson rank-order correlation (R), Spearman rank-order correlation (*ρ*), the difference between the 90th and 10th percentile data (P90), and the coefficient of divergence (*COD*).

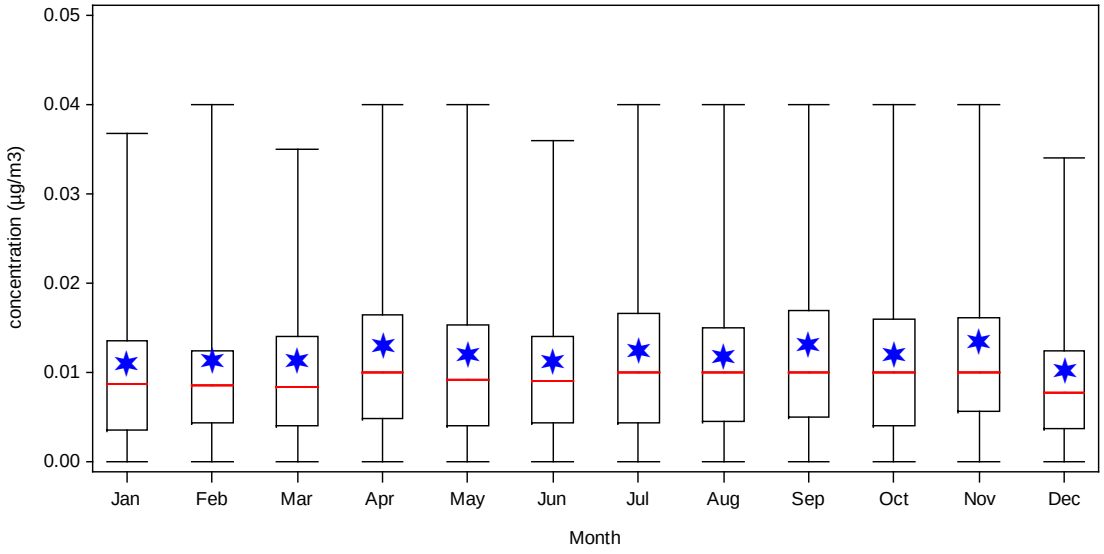
2.8.3 Seasonal Variation in Pb Concentrations

Monthly average Pb concentrations averaged over multiple sites and over 3 years from 2008-2010 are shown for Pb-TSP from source-oriented monitors (Figure 2-58), Pb-TSP from non-source-oriented monitors (Figure 2-59), Pb-PM_{10} (Figure 2-60), and Pb-$PM_{2.5}$ (Figure 2-61). For source-oriented Pb-TSP (Figure 2-58), monthly average concentrations were determined from between 146 and 154 samples in each month. For non-source-oriented TSP (Figure 2-59), monthly average concentrations were determined from between 141 and 151 samples in each month. A winter minimum was observed with December, January, and February exhibiting the three lowest monthly averages. In both cases, there is little seasonal variation. Minor variations in monthly averages are probably driven by exceptional events. Monthly median concentrations are very similar for all months.



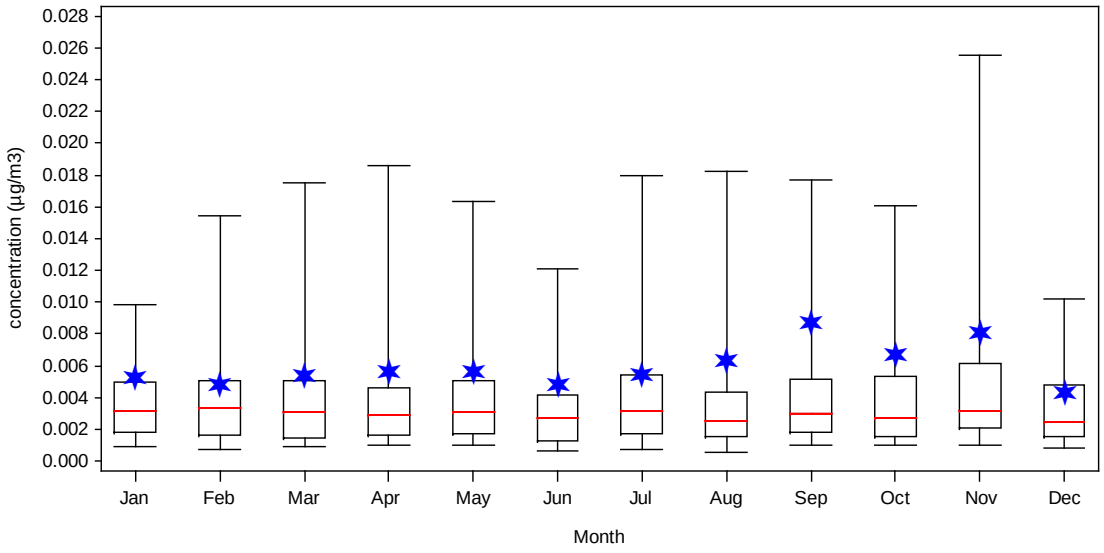
Note: Box and whisker plots are used for each month, with the box comprising the interquartile range of the data and the whiskers comprising the range within the 5th to 95th percentiles. The median is noted by the red line, and the blue star denotes the mean.

Figure 2-58 Monthly source-oriented Pb-TSP average (µg/m^3) over 12 months of the year, 2008-2010.



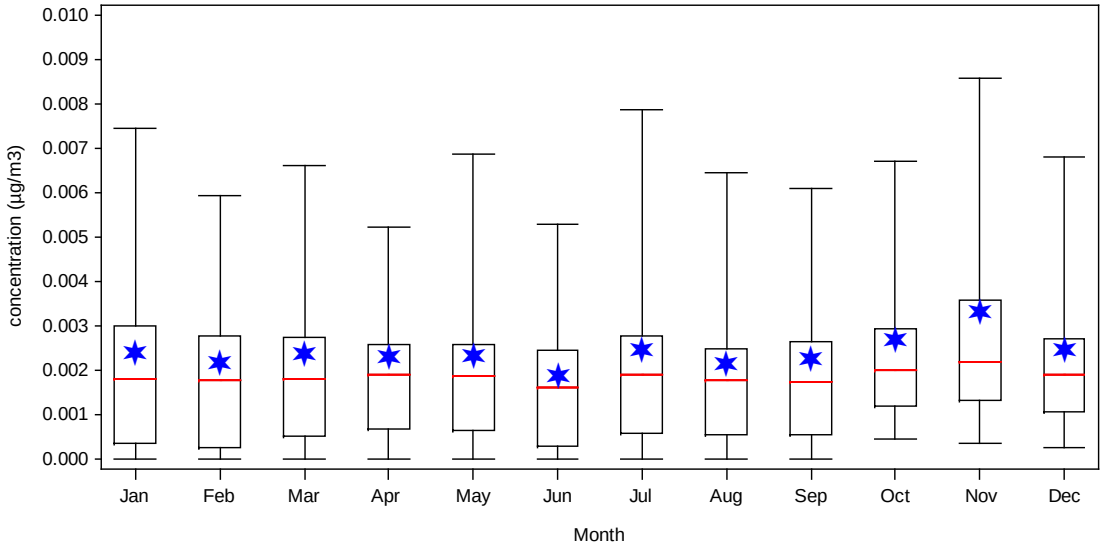
Note: Box and whisker plots are used for each month, with the box comprising the interquartile range of the data and the whiskers comprising the range within the 5th to 95th percentiles. The median is noted by the red line, and the blue star denotes the mean.

Figure 2-59 Monthly non-source-oriented Pb-TSP average (µg/m^3) over 12 months of the year, 2008-2010.



Note: Box and whisker plots are used for each month, with the box comprising the interquartile range of data and the whiskers comprising the range from 5th to 95th percentiles. The median is noted by the red line, and the blue star denotes the mean.

Figure 2-60 Monthly Pb-PM$_{10}$ average (µg/m^3) over 12 months of the year, 2007-2009.



Note: Box and whisker plots are used for each month, with the box comprising the interquartile range of the data and whiskers comprising the range from 5th to 95th percentiles. The median is noted by the red line, and the blue star denotes the mean.

Figure 2-61 Monthly Pb-$PM_{2.5}$ average (µg/m^3) over 12 months of the year, 2007-2009.

For both Pb-PM_{10} (Figure 2-60) and Pb-$PM_{2.5}$, (Figure 2-61) there is also little seasonal variation, with minor fluctuations in monthly averages probably driven by exceptional events, and similar monthly median concentrations for all months. Pb-PM_{10} monthly average concentrations were determined from between 100 and 109 samples and Pb-$PM_{2.5}$ from between 866 and 1,034 samples each month.

2.8.4 Size Distribution of Pb-bearing PM

Table 2-26 presents data for co-located Pb-TSP, Pb-PM_{10}, and/or Pb-$PM_{2.5}$ monitors. Table 2-27 contains metadata for studies in Section 2.5.3 involving size distribution data, and Table 2-28 contains the size distribution data for those studies. At times, the data were extracted from figures in the original references.

Table 2-26 Correlations and average of the concentration ratios for co-located monitors, TSP versus PM_{10}, TSP versus $PM_{2.5}$, and PM_{10} versus $PM_{2.5}$.

			Years	Corr	Avg Ratio	Years	Corr	Avg Ratio	Years	Corr	Avg Ratio
Site ID*	**CBSA**	**Land Type**	**PM_{10}: TSP**			**$PM_{2.5}$: TSP**			**$PM_{2.5}$: PM_{10}**		
060190008	Fresno, CA	Suburban	1995 - 2001	0.93	0.82	1992 - 2001	0.82	0.59	1995 - 2001	0.99	0.77
060190008	Fresno, CA	Suburban	1995 - 2001	0.93	0.83	1992 - 2001	0.80	0.56	1995 - 2001	0.96	0.82
060190008	Fresno, CA	Suburban							1995 - 2001	0.99	0.79
060190008	Fresno, CA	Suburban							1995 - 2001	0.98	0.77
060250004	El Centro, CA	Suburban							1995 - 1996	0.96	0.80
060250005	El Centro, CA	Suburban	1996 - 2001	0.79	0.98	1996 - 2001	0.77	0.73	1996 - 2001	0.99	0.71
060250005	El Centro, CA	Suburban	1996 - 2001	0.92	0.89	1996 - 2001	0.91	0.64			
060251003	El Centro, CA	Urban and Center City							1995 - 1995	0.81	0.72
060290004	Bakersfield, CA	Urban and Center City				1992 - 1994	0.90	0.55			
060290004	Bakersfield, CA	Urban and Center City				1992 - 1994	0.94	0.43			
060290014	Bakersfield, CA	Urban and Center City	1995 - 2000	0.94	0.75	1994 - 2000	0.96	0.51	1995 - 2000	0.96	0.71
060290014	Bakersfield, CA	Urban and Center City	1995 - 2000	0.92	0.78	1994 - 2000	0.92	0.53	1995 - 2000	0.91	0.77

Table 2-26 (Continued): Correlations and average of the concentration ratios for co-located monitors, TSP versus PM_{10}, TSP versus $PM_{2.5}$, and PM_{10} versus $PM_{2.5}$.

			Years	Corr	Avg Ratio	Years	Corr	Avg Ratio	Years	Corr	Avg Ratio
Site ID*	**CBSA**	**Land Type**	**PM_{10}: TSP**			**$PM_{2.5}$: TSP**			**$PM_{2.5}$: PM_{10}**		
060290014	Bakersfield, CA	Urban and Center City	1995 - 2000	0.47	0.80	1995 - 2000	0.28	0.60	1995 - 2000	0.84	0.80
060290014	Bakersfield, CA	Urban and Center City	1995 - 2000	0.43	0.81	1995 - 2000	0.27	0.62	1995 - 2000	0.98	0.72
060292004	Bakersfield, CA	Suburban							1995 - 2000	0.74	0.74
060310003	Hanford-Corcoran, CA	Unknown							1995 - 1998	0.97	0.83
060310004	Hanford-Corcoran, CA	Suburban							1996 - 2000	0.95	0.77
060370002	Los Angeles-Long Beach-Santa Ana, CA	Suburban							1995 - 2000	0.89	0.59
060374002	Los Angeles-Long Beach-Santa Ana, CA	Suburban	1995 - 2000	0.76	0.38	1992 - 2000	0.62	0.30	1995 - 2000	0.91	0.62
060374002	Los Angeles-Long Beach-Santa Ana, CA	Suburban	1995 - 2000	0.87	0.72	1992 - 2000	0.43	0.44			
060390001	Madera-Chowchilla, CA	Urban and Center City							1995 - 1996	0.98	0.90
060631008	NONE (Plumas Co., CA)	Unknown							1997 - 1999	0.95	0.72
060658001	Riverside-San Bernardino-Ontario, CA	Suburban	1995 - 1996	0.13	0.39	1992 - 1996	0.31	0.33	1995 - 1997	0.94	0.67

Table 2-26 (Continued): Correlations and average of the concentration ratios for co-located monitors, TSP versus PM_{10}, TSP versus $PM_{2.5}$, and PM_{10} versus $PM_{2.5}$.

			Years	Corr	Avg Ratio	Years	Corr	Avg Ratio	Years	Corr	Avg Ratio
Site ID*	**CBSA**	**Land Type**	**PM_{10}: TSP**			**$PM_{2.5}$: TSP**			**$PM_{2.5}$: PM_{10}**		
060658001	Riverside-San Bernardino-Ontario, CA	Suburban	1995 - 1997	0.93	0.72	1992 - 1997	0.86	0.46			
060670010	Sacramento--Arden-Arcade--Roseville, CA	Urban and Center City							1995 - 2001	0.99	0.75
060710014	Riverside-San Bernardino-Ontario, CA	Suburban							1996 - 2000	0.78	0.73
060771002	Stockton, CA	Urban and Center City	1995 - 2000	0.70	0.84	1992 - 2000	0.59	0.56	1995 - 2000	0.94	0.71
060771002	Stockton, CA	Urban and Center City	1995 - 2000	0.91	0.74	1992 - 2000	0.76	0.48			
060850004	San Jose-Sunnyvale-Santa Clara, CA	Urban and Center City	1995 - 2000	0.87	0.63	1994 - 1997	0.11	0.36	1995 - 2000	0.95	0.69
060850004	San Jose-Sunnyvale-Santa Clara, CA	Urban and Center City				1992 - 1993	0.42	0.37			
060850004	San Jose-Sunnyvale-Santa Clara, CA	Urban and Center City				1992 - 2000	0.54	0.39			
060990002	Modesto, CA	Urban and Center City	1995 - 1998	0.96	0.79	1992 - 1998	0.24	0.61	1995 - 1998	0.64	0.80
060990002	Modesto, CA	Urban and Center City							1997 - 1998	0.50	0.73
060990005	Modesto, CA	Urban and Center City							1998 - 2000	0.99	0.71
060990005	Modesto, CA	Urban and Center City							1998 - 2000	0.97	0.71
061072002	Visalia-Porterville, CA	Urban and Center City							1995 - 2000	0.99	0.70

Table 2-26 (Continued): Correlations and average of the concentration ratios for co-located monitors, TSP versus PM_{10}, TSP versus $PM_{2.5}$, and PM_{10} versus $PM_{2.5}$.

			Years	Corr	Avg Ratio	Years	Corr	Avg Ratio	Years	Corr	Avg Ratio
Site ID*	**CBSA**	**Land Type**	**PM_{10}: TSP**			**$PM_{2.5}$: TSP**			**$PM_{2.5}$: PM_{10}**		
170310022	Chicago-Naperville-Joliet, IL-IN-WI	Suburban	1992	0.81	0.94						
170310052	Chicago-Naperville-Joliet, IL-IN-WI	Suburban	1992	0.84	0.86						
171190010	St. Louis, MO-IL	Urban and Center City	1992	0.40	0.96						
171191007	St. Louis, MO-IL	Urban and Center City	1992	0.92	0.91						
171630010	St. Louis, MO-IL	Suburban	1992	0.96	0.91						
180890023	Chicago-Naperville-Joliet, IL-IN-WI	Urban and Center City	2007 - 2008	0.73	0.86						
201730007	Wichita, KS	Suburban	1990 - 1997	0.18	1.28						
201730008	Wichita, KS	Suburban	1990 - 1997	0.34	1.12						
201730009	Wichita, KS	Suburban	1990 - 1997	0.54	1.05						
201731012	Wichita, KS	Suburban	1990 - 1997	0.85	0.89						
201770007	Topeka, KS	Urban and Center City	1990 - 1997	0.56	0.99						
202090015	Kansas City, MO-KS	Urban and Center City	1990 - 1997	0.75	0.80						
202090020	Kansas City, MO-KS	Urban and Center City	1990 - 1997	0.99	0.81						
270530053	Minneapolis-St. Paul-Bloomington, MN-WI	Urban and Center City	1996 - 2001	0.54	0.57						
300490719	Helena, MT	Suburban	1990 - 1991	0.81	0.48						
300490719	Helena, MT	Suburban	1990	0.79	0.49						

Table 2-26 (Continued): Correlations and average of the concentration ratios for co-located monitors, TSP versus PM_{10}, TSP versus $PM_{2.5}$, and PM_{10} versus $PM_{2.5}$.

			Years	Corr	Avg Ratio	Years	Corr	Avg Ratio	Years	Corr	Avg Ratio
Site ID*	**CBSA**	**Land Type**	**PM_{10}: TSP**			**$PM_{2.5}$: TSP**			**$PM_{2.5}$: PM_{10}**		
450430001	Georgetown, SC	Urban and Center City	1990 - 1991	0.71	0.60						
450790014	Columbia, SC	Suburban	1990	0.82	0.89						
450791003	Columbia, SC	Urban and Center City	1990	0.90	0.94						
450791003	Columbia, SC	Urban and Center City	1990	0.90	0.83						

*Note: For comparability, comparisons were limited to samples from sites which had at least 30 pairs of co-located samples, with both samples above the MDL and where both monitors reported data at STP.

Table 2-27 Metadata for studies of Pb-PM size distribution.

Reference	Location	Nearest source	Proximity to source	Sampling dates
Yi et al. (2006)	Jersey City, NJ- an urban/industrial area	Jersey City- near Manhattan, NJ Turnpike, Hudson River- high gas/oil consumption for industry/domestic heating, heavy gasoline & diesel powered vehicles and ship traffic from harbor.	Close	ASD measurements: June 10-20, 2002
	New Brunswick, NJ- a suburban area	New Brunswick- near NJ Parkway and Garden State Parkway		
Bein et al. (2006)	Pittsburg, PA	Article does not describe sources; other articles also use data from Pittsburgh Air Quality Study (PAQS) and may have more info on sources	-	July 2001-September 2002
Pekey et al. (2010)	Kocaeli, Turkey	Kocaeli is a very industrialized and urbanized region in Turkey; sources include a large refinery, a petrochemical complex, a hazardous waste incinerator and industry operations for textile, machine, mine, metal, food, automotive, paper, chemistry, wood, petroleum, tanning, and coal sectors, plus heavy traffic	-	-
Singh et al. (2002)	Downey, CA- a city in Los Angeles County along "Alameda corridor" joining coastal area to downtown LA	Downey- a "source" site affected by fresh PM emissions from nearby oil refineries, industry, and heavy diesel emissions	Downey: 10 km downwind of refineries; 2-4 km from Interstates I-710 and I-605	Downey: September 2000-January 2001
	Riverside, CA- an inland county east of LA	Riverside- a "receptor" site affected by aged PM emissions including high vehicle emissions in LA	Riverside- 70 km east of downtown LA	Riverside: February 2001-June 2001
Dall'Osto et al. (2008)	U.K. national air quality monitoring station in Port Talbot, U.K.	One of the U.K.'s largest integrated steelworks; near major roadways; (Table 1 of this study describes the plants, operations, emission types, and emission components for steelwork sources)	Monitoring site next to steelwork	April 24-May 5, 2006

Table 2-27 (Continued): Metadata for studies of Pb-PM size distribution.

Reference	Location	Nearest source	Proximity to source	Sampling dates
Weitkamp et al. (2005)	Coking facility near Pittsburgh, PA	Large coking facility that converts 6 million tons of coal to 4 million tons of metallurgic coke every year	Sampling site downwind, directly across river from coke facility(~400m)	August 22-September 5, 2002
Pekey et al. (2010)	Indoor/outdoor sample points for 15 homes in Kocaeli, Turkey	Kocaeli is a very industrialized and urbanized region in Turkey; sources include a large refinery, a petrochemical complex, a hazardous waste incinerator and industry operations for textile, machine, mine, metal, food, automotive, paper, chemistry, wood, petroleum, tanning, and coal sectors, plus heavy traffic	15 Kocaeli homes chosen as representative sample; 10 close to high traffic roads, 5 near low/moderate traffic roads	Summer: May 31-June 29, 2006 Winter: December 16-January 20, 2007
Sabin et al. (2006b)	Near Interstate I-405 between Sunset Blvd and Wilshire Blvd, Los Angeles, CA	Heavy traffic on Interstate I-405 Freeway	UP: 150m upwind of Interstate I-405 (background) DW1: 10m downwind DW2: 150m downwind DW3: 450m downwind	April 13-May 1, 2004
Song and Gao (2011)	Carlstadt, NJ	Heavy traffic on NJ Turnpike	~5m from roadside of the highway	Winter: December 2007-February 2008 Summer: July 2008
Zereini et al. (2005)	3 Sites in Frankfurt, Germany with different traffic densities	Vehicle emissions	Site 1: next to main street with 32,500 cars/day Site 2: next to side street with <1,000 cars/day Site 3: large garden 8km NW of city	August 2001 to July 2002
Lough et al. (2005)	Two road traffic tunnels in Milwaukee, WI	Traffic emissions	5 m upwind from entrance (inside tunnel); 15 m upwind from tunnel exit	Summer: July 31-August 28, 2000 Winter: December 13-January 17, 2001
Hays et al. (2011)	20m downwind of Interstate I-440 highway in Raleigh, NC	Traffic emissions from highway	20 m downwind	July 26-31 and August 3-10, 2006

Table 2-27 (Continued): Metadata for studies of Pb-PM size distribution.

Reference	Location	Nearest source	Proximity to source	Sampling dates
Chen et al. (2010b)	Roadside site and site inside highway tunnel in Taipei, Taiwan	Vehicle emissions	Roadside: sidewalk 4m from road Tunnel: relay station in tunnel 1.4km from outlet; 2 m from traffic lane	January to December 2008
Birmili et al. (2006)	Remote background: Mace Head atmospheric research station in Connemara, Ireland	Traffic emissions	Remote background:	Remote background: August 8-28, 2002
	Urban background: University of Birmingham campus, U.K.		Urban background: at least 100m from road traffic	Urban background: April 23-October 7, 2002
	Roadside: A38 Bristol Road, Birmingham, U.K.		Roadside: 4 m from traffic	Roadside: July 8-12, 2002
	Road Tunnel: Queensway Underpass in Birmingham, U.K.		Road Tunnel: 30 m from tunnel exit	Road Tunnel: July 2, 2002
Bruggemann et al. (2009)	Roadside in Dresden, Germany	Traffic emissions from busy street (traffic density~55,000 per day; 8% trucks), tramline, railway station	Next to road, near tramline crossing, 200 m to railway station	September 2003-August 2004
Harrison et al. (2003)	Roadside in Birmingham, U.K.	Traffic emissions from A38	9m from road	October 26, 2000 to January 17, 2001
Wang et al. (2006d)	Suburb of Kanazawa, Japan- a western coastal city; the largest in Hokuriku region of Japan	Emissions from road traffic, nearby incinerators and electricity generation plants, and sea salt	Next to road; ~5km from incinerators and electricity generation plants; situated on west coast	May-June 2003
Martuzevicius et al. (2004)	9 Locations in Cincinnati, Ohio metropolitan area	Vehicle emissions from Cincinnati highway network; emission from industry (233 facilities within municipal area limits)	11 Sites- varying distance to linear and point sources; distance to major highways ranges from 210 m to 4,590 m	December 2001-November 2002

Table 2-27 (Continued): Metadata for studies of Pb-PM size distribution.

Reference	Location	Nearest source	Proximity to source	Sampling dates
Moreno et al. (2008)	3 Sampling sites in Mexico City (Mexico) Metropolitan Area: 1 Site in the industrial center (T0), 1 Site NE outside city limits (T1), and 1 Rural site north of city (T2)	Urban pollution sources- traffic emissions, industry	3 Sites with varying relation to "Mexico urban plume" by distance, wind direction	March 2006
Goforth and Christoforou (2006)	Lake Hartwell, GA (rural southeast U.S.)	-	-	February-March 2003
Makkonen et al. (2010)	Virolahti EMEP station, Finland	European Route E18 (3,000 vehicles/day)	5 km	August 2007
Wojas and Almquist (2007)	Oxford, OH and other towns in Greater Cincinnati region	Transportation, manufacturing processes, and coal-fired power plants	~80 km to northwest Cincinnati	January to December 2005
Lin et al. (2005)	Roadside in city in southern Taiwan	Traffic emissions (avg traffic load = 72,000 vehicles/day), Pingtung Industrial Park (146 factories), Nearby crematory	10 m from road, 2 km from industrial park (146 factories, [i.e., electron apparatus, metal, and food manufacturing]), 1 km from crematory	February to April 2004
Csavina et al. (2011)	Main sampling site in Winkelman, Arizona at Hayden High School	Nearby an active mining and smelting site in Hayden, Arizona	2km from mine tailings pile; 1km from smelting operations, main smoke stack, and slag pile	December 2008-November 2009

Table 2-27 (Continued): Metadata for studies of Pb-PM size distribution.

Reference	Location	Nearest source	Proximity to source	Sampling dates
Fang and Huang (2011)	3 Sites in central Taiwan: A school (Hung-kuang), A wetland (Gaomei in Taichung), An industrial site (Quan-xing)	Emissions from vehicle traffic and industry	- Hung-kuang (HK): in residential area 2 km from major expressway Gaomei in Taichung (GM): 300 hectare wetland with coal combustion-based Taichung Thermal Power Plant (located along the coast of the west side of the sampling site) Quan-xing (QX): town with lots of industry including metal manufacturing, textiles, petroleum and coal products	November 2010-December 2010
Lim et al. (2006)	7 Sites around Los Angeles, CA, including 6 urban watershed sites and one non-urban coastal watershed.	Not stated	-	August, 2002 – June, 2003

Table 2-28 Size distribution data for various studies described in Table 2-27.

Reference	Location	Size Bin	Concentration
Yi et al. (2006)	Jersey City, NJ	0.18-0.32	0.001054
		0.32-0.56	0.000668
		0.56-1	0.000952
		1-1.8	0.000852
		1.8-3.2	0.000609
		3.2-5.6	0.001229
		5.6-10	0.001591
		10-14.4	0.000948
		14.4-19.9	0.000171
		19.9-26.1	0.000693
		26.1-36.1	0.000333
		36.1-100	0.000447
Yi et al. (2006) (Continued)	New Brunswick, NJ	0.18-0.32	0.001146
		0.32-0.56	0.00078
		0.56-1	0.001733
		1-1.8	0.001083
		1.8-3.2	0.000373
		3.2-5.6	0.000446
		5.6-10	0.000347
		10-14.4	0.000182
		14.4-19.9	2.02E-09
		19.9-26.1	1.35E-05
		26.1-36.1	1.62E-05
		36.1-100	0.000152

Table 2-28 (Continued): Size distribution data for various studies described in Table 3-27.

Reference	Location	Size Bin	Concentration
Bein et al. (2006)	Pittsburgh, PA	1-1.8	0.096608
		1.8-3.2	0.314846
		3.2-5.6	0.187393
		5.6-10	0.239094
Singh et al. (2002)	Downey, CA	<0.1 µm	0.00133
		0.1-0.35	0.00419
		0.35-1.0	0.00334
		1.0-2.5	0.00189
		2.5-10	0.00175
	Riverside, CA	<0.1 µm	0.0004
		0.1-0.35	0.00089
		0.35-1.0	0.0018
		1.0-2.5	0.001
		2.5-10	0.00297
Dall'Osto et al. (2008)	Port Talbot, U.K.	0.1-0.196	0.000211
		0.196-0.356	0.001871
		0.356-0.57	0.005424
		0.57-1	0.004935
		1-1.8	0.010229
		1.8-3.1	0.002216
		3.1-6.2	0.001847
		6.2-9.9	0.000807
		9.9-18	0.000141
Sabin et al. (2006b)	Los Angeles, CA, 10 m downwind of road	<6	0.007953
		6-11	0.004172
		11-20	0.00013
		20-29	0.000522
		>29	0.004563

Table 2-28 (Continued): Size distribution data for various studies described in Table 3-27.

Reference	Location	Size Bin	Concentration
Zereini et al. (2005)	Frankfurt, Germany	<0.43	0.005904
	Main street; Frankfurt	0.43-0.63	0.005332
		0.63-1.1	0.004285
		1.1-2.1	0.002857
		2.1-3.3	0.002666
		3.3-4.7	0.002857
		4.7-5.8	0.001809
		5.8-9.0	0.002476
		>9.0	0.004285
Zereini et al. (2005) (Continued)	Side street, Frankfurt	<0.43	0.00332
		0.43-0.63	0.002818
		0.63-1.1	0.002239
		1.1-2.1	0.001544
		2.1-3.3	0.000849
		3.3-4.7	0.000772
		4.7-5.8	0.000386
		5.8-9.0	0.00054
		>9.0	0.000733
Zereini et al. (2005) (Continued)	Rural background, Frankfurt	<0.43	0.003312
		0.43-0.63	0.002442
		0.63-1.1	0.002208
		1.1-2.1	0.001405
		2.1-3.3	0.000602
		3.3-4.7	0.000502
		4.7-5.8	0.000201
		5.8-9.0	0.000201
		>9.0	0.000502

Table 2-28 (Continued): Size distribution data for various studies described in Table 3-27.

Reference	Location	Size Bin	Concentration
Hays et al. (2011)	Raleigh, NC	0.03-0.06	0.000186
		0.06-0.108	0.000395
		0.108-0.17	0.000732
		0.17-0.26	0.001486
		0.26-0.4	0.003593
		0.4-0.65	0.007315
		0.65-1	0.00423
		1-1.6	0.002719
		1.6-2.5	0.002701
		2.5-4.4	0.003346
		4.4-6.8	0.00123
		6.8-10	0.000934
		10-18	0.001265
Chen et al. (2010b)	Taipei, Taiwan tunnel	<0.1	0.018409
		0.1-2.5	0.019773
		2.5-10	0.030682
		<0.1	0.00125
		0.1-2.5	0.020625
		2.5-10	0.024375
Bruggemann et al. (2009)	Dresden, Germany	0.05-0.14	0.001078
	Summer; Dresdeb	0.14-0.42	0.002874
		0.42-1.2	0.004671
		1.2-3.5	0.001617
		3.5-10	0.000539
Bruggemann et al. (2009) (Continued)	Winter; Dresden	0.05-0.14	0.002335
		0.14-0.42	0.00521
		0.42-1.2	0.013293
		1.2-3.5	0.003054
		3.5-10	0.000539

Table 2-28 (Continued): Size distribution data for various studies described in Table 3-27.

Reference	Location	Size Bin	Concentration
Harrison et al. (2003)	Birmingham, U.K.	<0.2	0.00685
		0.2-1	0.014923
		1-2	0.002446
		2-10	0.00318
		>10	0.000489
Wang et al. (2006d)	Kanazawa, Japan	0.1-0.43	0.000792
		0.43-0.65	0.000748
		0.65-1.1	0.00118
		1.1-2.1	0.00103
		2.1-3.3	0.000393
		3.3-4.7	0.000678
		4.7-7	0.000375
		7-11	0.000229
		11-18	0.000125
Martuzevicius et al. (2004)	Cincinnati, OH	~0.1-0.18	0.000758
	Cycle VIII; Cincinnati	0.18-0.32	0.002045
		0.32-0.56	0.003258
		0.56-1	0.00447
		1-1.8	0.005758
		1.8-3.2	0.00697
		3.2-5.6	0.008333
		5.6-10	0.009545
Martuzevicius et al. (2004) (Continued)	Cycle IX; Cincinnati	~0.1-0.18	0.000455
		0.18-0.32	0.001591
		0.32-0.56	0.002879
		0.56-1	0.004091
		1-1.8	0.005379
		1.8-3.2	0.006667
		3.2-5.6	0.007879

Table 2-28 (Continued): Size distribution data for various studies described in Table 3-27.

Reference	Location	Size Bin	Concentration
		5.6-10	0.009091
Lim et al. (2006) Note: Summer and Fall data not provided because there is more uncertainty in the sites for those datasets.	Los Angeles, CA	6-11	1.315
	Los Angeles River Watershed #1	11-20	0.743
	Winter	20-29	-
		>29	0.821
	Spring	6-11	2.302
		11-20	1.212
		20-29	-
		>29	1.485
Lim et al. (2006) (Continued) Note: Summer and Fall data not provided because there is more uncertainty in the sites for those datasets.	Los Angeles River Watershed #2	6-11	3.025
	Winter	11-20	1.251
		20-29	0.547
		>29	1.212
	Spring	6-11	1.042
		11-20	1.212
		20-29	0.312
		>29	0.782
Lim et al. (2006) (Continued) Note: Summer and Fall data not provided because there is more uncertainty in the sites for those datasets.	Los Angeles River Watershed #3	6-11	1.116
	Winter	11-20	1.055
		20-29	0.547
		>29	1.016
	Spring	6-11	2.299
		11-20	1.055
		20-29	0.508
		>29	1.524

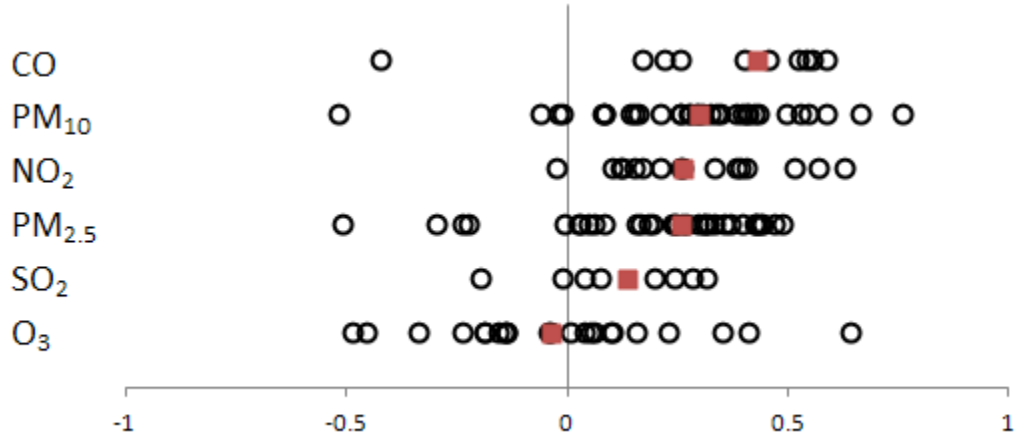
Table 2-28 (Continued): Size distribution data for various studies described in Table 3-27.

Reference	Location	Size Bin	Concentration
Lim et al. (2006) (Continued) Note: Summer and Fall data not provided because there is more uncertainty in the sites for those datasets.	Santa Ana River Watershed	6-11	-
	Winter	11-20	0.097
		20-29	0.235
		>29	0.195
	Spring	6-11	0.185
		11-20	0.235
		20-29	0.039
		>29	0.156
Lim et al. (2006) (Continued) Note: Summer and Fall data not provided because there is more uncertainty in the sites for those datasets.	Ballona Creek Watershed	6-11	1.263
	Winter	11-20	1.016
		20-29	0.313
		>29	0.664
	Spring	6-11	5.064
		11-20	1.29
		20-29	0.312
		>29	2.58
Lim et al. (2006) (Continued) Note: Summer and Fall data not provided because there is more uncertainty in the sites for those datasets.	Dominguez Creek Watershed	6-11	2.315
	Winter	11-20	1.368
		20-29	0.547
		>29	0.625
	Spring	6-11	0.683
		11-20	0.469
		20-29	0.078
		>29	0.508

Table 2-28 (Continued): Size distribution data for various studies described in Table 3-27.

Reference	Location	Size Bin	Concentration
Lim et al. (2006) (Continued) Note: Summer and Fall data not provided because there is more uncertainty in the sites for those datasets.	Malibu Creek (non-urban)	6-11	0.201
	Winter	11-20	0.391
		20-29	-
		>29	0.039
	Spring	6-11	0.211
		11-20	0.156
		20-29	-
		>29	0.117

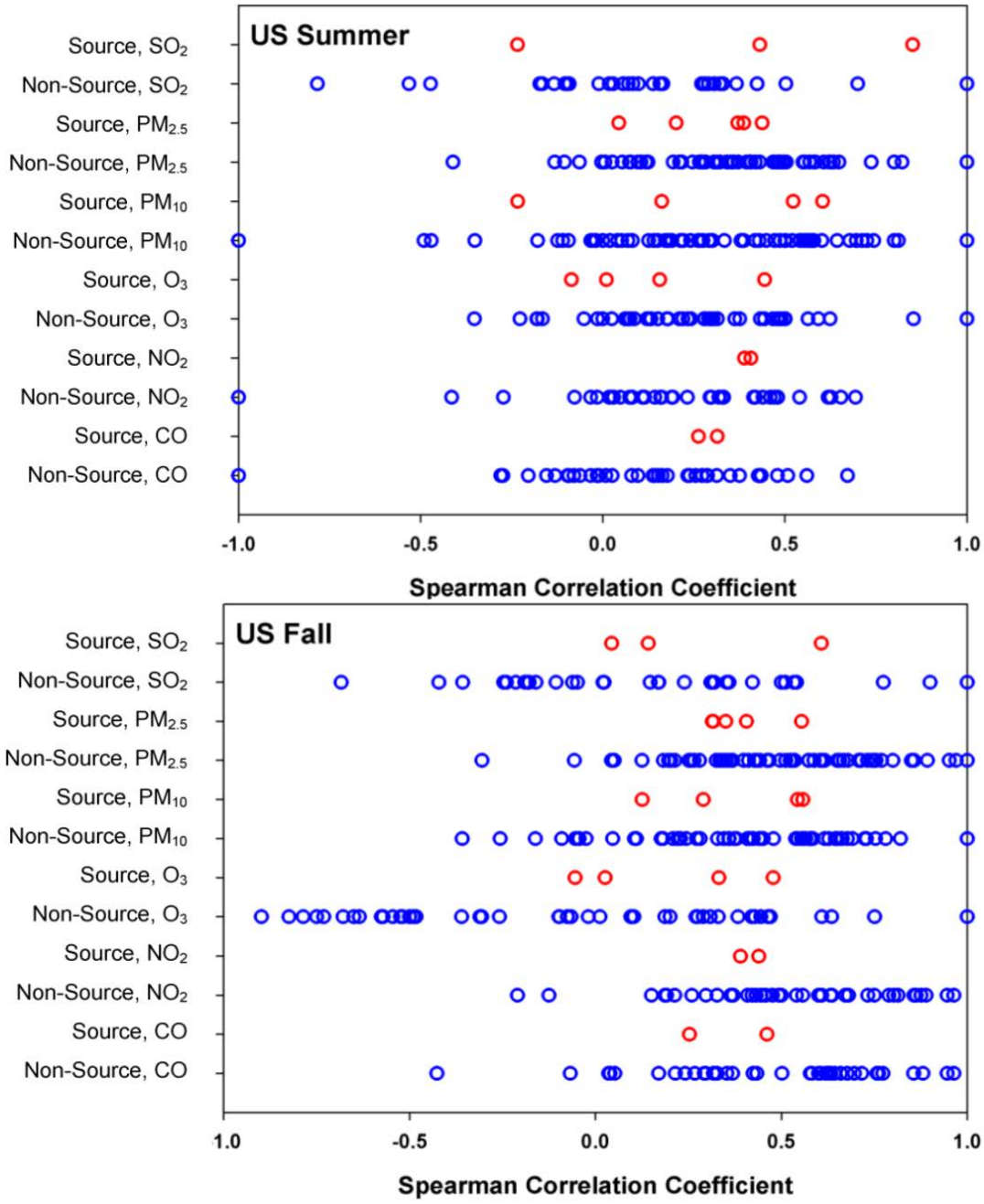
2.8.5 Pb Concentration in a Multipollutant Context



Note: Correlations were calculated from available data when data were above MDL and there were at least 30 data pairs available for comparison.

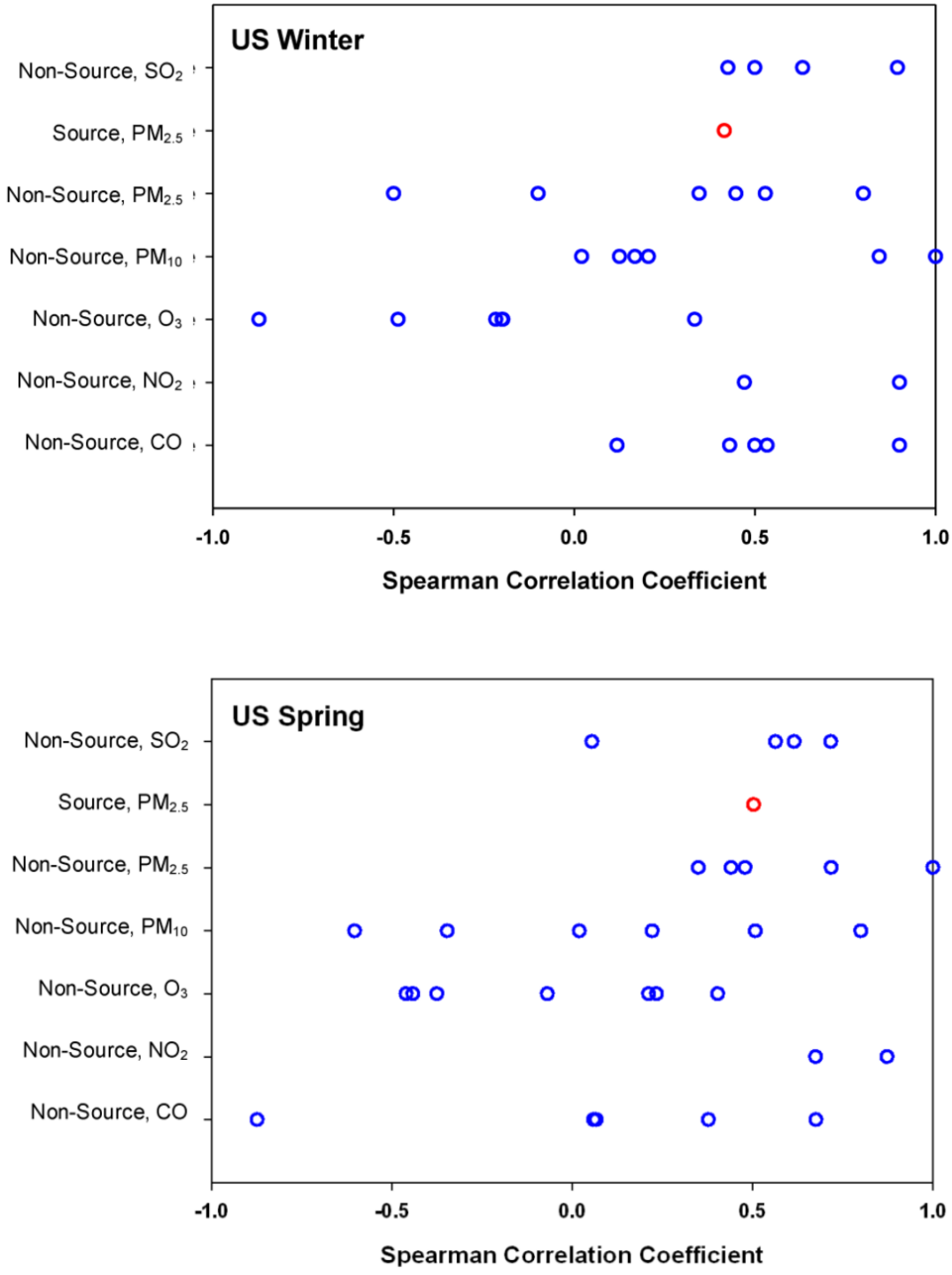
Correlations for individual sites are shown with black open circles, while median correlations are illustrated with a red square.

Figure 2-62 Spearman correlations of monitored non-source Pb-TSP concentration with daily averages of copollutant concentrations, 2008-2010.



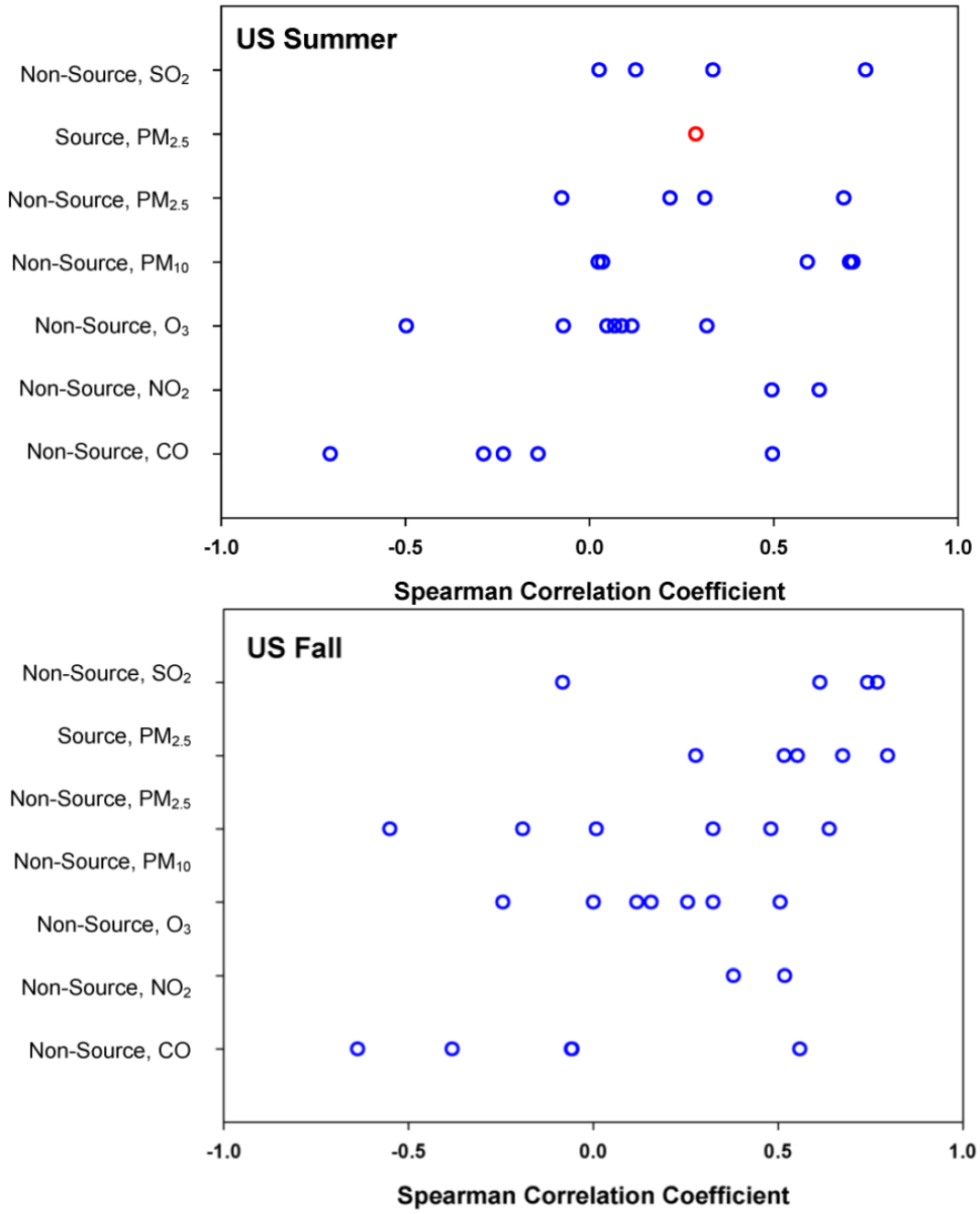
Note: Top panel: Summer; Bottom panel: Fall.

Figure 2-63 Seasonal correlations of monitored Pb-TSP concentration with copollutant concentrations, 2007-2008.



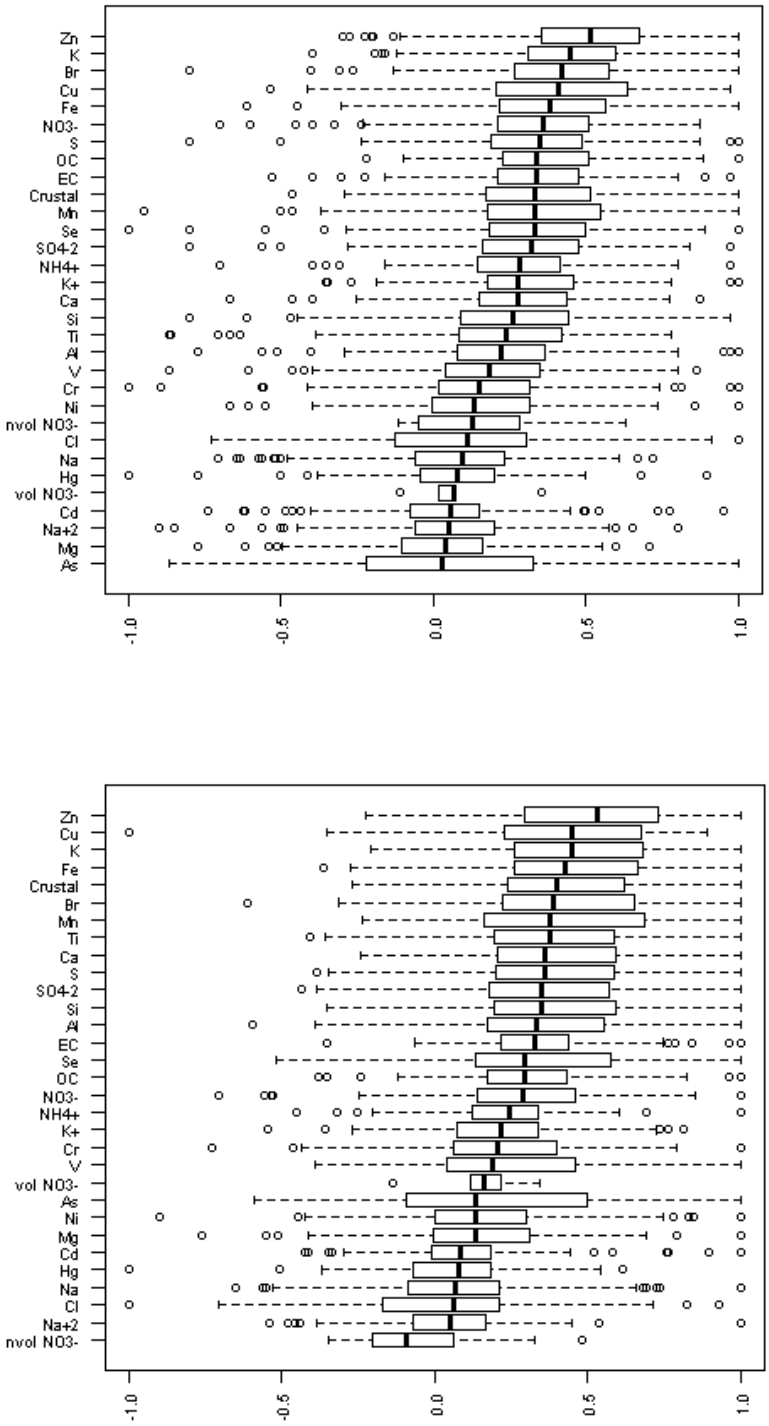
Note: Top panel: Winter; Bottom panel: Spring.

Figure 2-64 Seasonal correlations of monitored Pb-TSP concentration with copollutant concentrations, 2009.



Note: Top panel: Summer; Bottom panel: Fall.

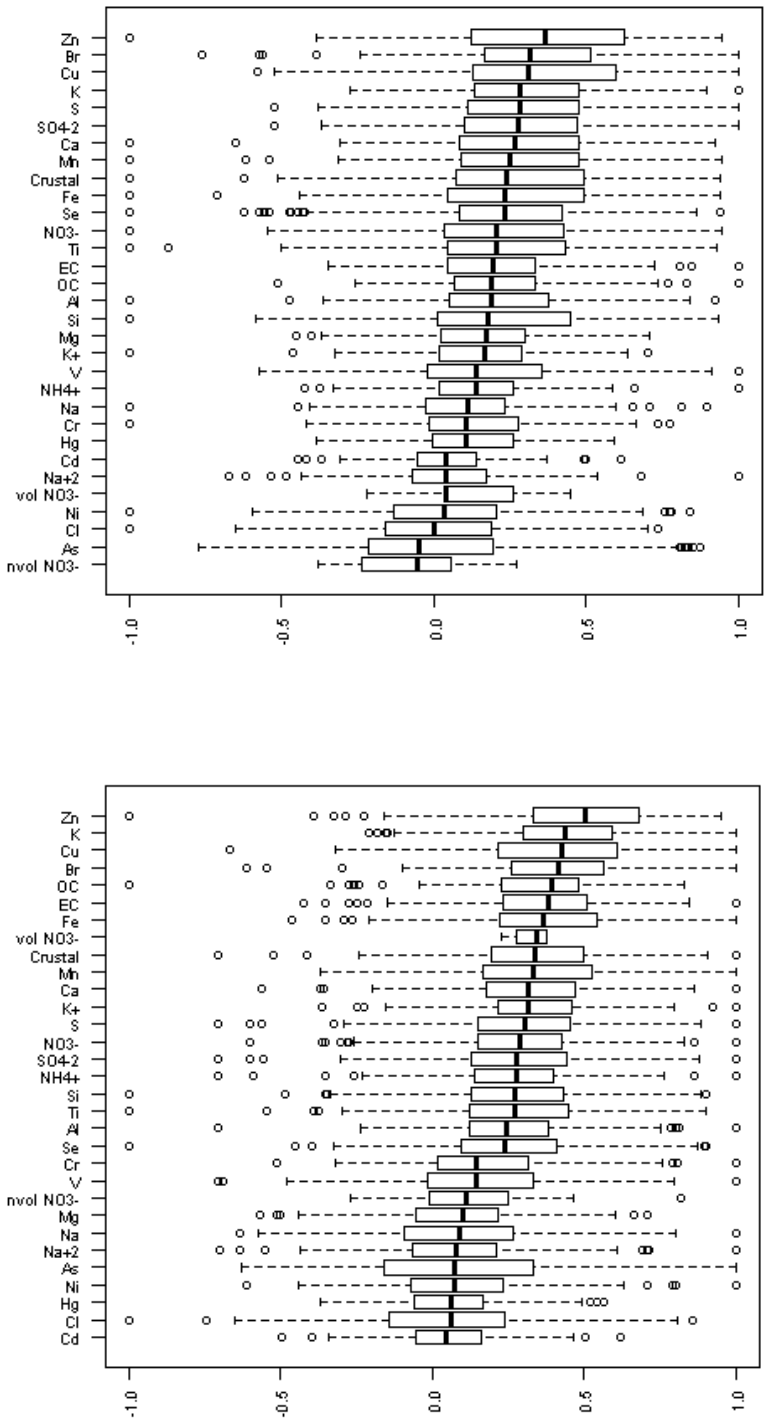
Figure 2-65 Seasonal correlations of monitored Pb-TSP concentration with copollutant concentrations, 2009.



Top panel: Winter; Bottom panel: Spring.

Note: “nvol” = non-volatile, “vol” = volatile, and organic carbon (OC) samples were blank-adjusted.

Figure 2-66 Seasonal correlations of monitored Pb-$PM_{2.5}$ concentration with copollutant concentrations, 2007-2009.



Top panel: Summer; Bottom panel: Fall.
Note: "nvol" = non-volatile, "vol" = volatile, and organic carbon (OC) samples were blank-adjusted.

Figure 2-67 Seasonal correlations of monitored Pb-$PM_{2.5}$ concentration with copollutant concentrations, 2007-2009.

Table 2-29 Copollutant exposures for various trace metal studies.

	Adgate et al. (2007)		Riediker et al. (2003)		Pekey et al. (2010)	Molnar et al. (2007)		
	I-R (med)[a,b]	Personal (median)[c]	Vehicle (range)[c]	Roadside (range)[c]	I –near industry (range)[a]	I-R (median)[a,b]	I-School (median)[a]	I-Pre-School (median)[a]
Location	**Minnesota, U.S.**		**New Jersey, U.S.**		**Kocaeli, Turkey**	**Stockholm, Sweden**		
$PM_{2.5}$			24,000	31,579	24,400-29,800			
Pb	1.5	3.2	2-3	4-6	34-85	2.8	2.5	1.7
S	272.1	351.6	905-1,592	1,416-2,231	435-489	330	290	220
Ca	85.0	174.1	31-44	18-40	309-452	70	110	58
Al	23.3	58.6			53-60			
Na	20.6	31.9						
Fe	43.1	78.6	307-332	82-163	44-58	57	100	71
Mg	16.3	27.5						
K	38.4	47.5	6-75	23-57	160-215	120	96	67
Ti	0.8	1.4	9-10	6-10	29-39	8.0	13	8.7
Zn	6.5	9.6	5-10	14-17	51-88	14	17	11
Cu	1.-0.15	4.9	18-32	8-16	21-58	9.3	1.7	2.1
Ni	2.4	1.8	0	0	2-3	0.99	1.0	0.72
Mn	0.21	2.3	3-4	3	28-32	2.2	2.5	2.1
Sb	0.12	0.30						
Cd	0.12	0.14	4-6	4-7				
V	0.05	0.16	1	1	3-5	2.5	2.7	1.8
La	0.00	0.11						
Cs	0.00	0.00						
Th	0.00	0.00						

Table 2-29 (Continued): Copollutant exposures for various trace metal studies.

	Adgate et al. (2007)		Riediker et al. (2003)		Pekey et al. (2010)	Molnar et al. (2007)		
Sc	0.00	0.01						
Ag	0.07	0.08						
Co	0.02	0.07						
Cr	1.2	2.6	2	1	3-8	<1.1	1.3	1.1
Si			198-464	338-672	387-401			
Cl			7-32	3-9				
Se			1	1-2				
Rb			1	1				
Sr			5-28	1				
As			1	1	1-2			
Mo								
Br						2.1	1.3	1.3

[a]I: Indoor; Units: ng/m^3

[b]R: Residential; Units: ng/m^3

[c]Units: ng/m^3

References for Chapter 2

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CHAPTER 3 EXPOSURE, TOXICOKINETICS, AND BIOMARKERS

3.1 Exposure Assessment

The purpose of this section is to present recent studies that provide insight about human exposure to Pb through various pathways. Pb is considered to be a multimedia contaminant with multiple pathways of exposure. The relative importance of various media in affecting Pb exposure changes with source strength and location, location and time activity of the exposed individuals, behavior of the exposed individuals, and risk factors such as age and socioeconomic factors (risk factors are discussed in detail in Chapter 3). Blood Pb and bone Pb biomarkers (discussed in Section 3.4 and Section 3.5), are often used to indicate composite Pb exposure resulting from multiple media and pathways of exposure.

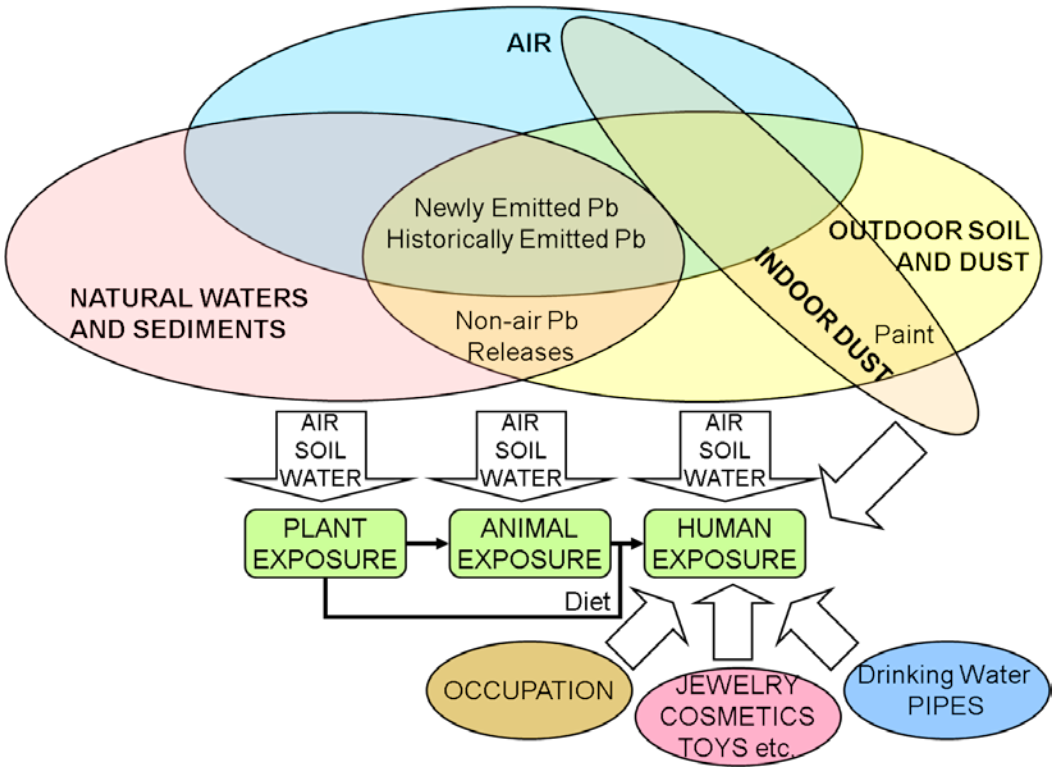
The recent information provided here builds upon the conclusions of the 2006 Pb AQCD (U.S. EPA, 2006b), which found that air Pb concentrations and blood Pb levels have decreased substantially following the restrictions on Pb in on-road vehicle gasoline, Pb in household paints, the use of Pb solder, and reductions in industrial Pb emissions that have occurred since the late 1970s. Nevertheless, detectable quantities of Pb have still been observed to be bioaccessible in various media types. It was reported in the 2006 Pb AQCD (U.S. EPA, 2006b) that airborne maximum quarterly Pb concentrations in the U.S. were in the range of 0.03-0.05 μg/m^3 for non-source-oriented monitors for the years 2000-2004 and were 0.10-0.22 μg/m^3 for source-oriented monitors during that time period, while blood Pb levels reached a median of 1.70 μg/dL among children (1-5 years of age) in 2001-2002. It was also observed that Pb exposures were associated with nearby industrial Pb sources, presence of Pb-based paint, and Pb deposited onto food in several of the studies described in the 2006 Pb AQCD.

3.1.1 Pathways for Pb Exposure

Pathways of Pb exposure are difficult to disentangle because Pb has multiple sources in the environment and passes through various environmental media. These issues are described in detail in Sections 2.2 and 2.3. Air-related pathways of Pb exposure are the focus of this ISA. Pb can be emitted to air, soil, or water and then cycle through any or all of these media. In addition to primary emission of particle-bound or gaseous Pb to the atmosphere, Pb can be resuspended to the air from soil or dust. Additionally, Pb-bearing PM can be deposited from the air to soil or water through wet and dry deposition. Air-

related Pb exposures also include inhalation and ingestion of Pb-contaminated food, water or other materials following atmospheric deposition of Pb; these exposures include dust and soil via hand-to-mouth contact. In general, air-related pathways include those pathways where Pb passes through ambient air on its path from a source to human exposure. Some non-air-related exposures of Pb include ingestion of indoor Pb paint, Pb in diet as a result of inadvertent additions during food processing, and Pb in drinking water attributable to Pb in distribution systems, as well as other generally less prevalent pathways.

The complicated nature of Pb exposure is illustrated in Figure 3-1, in which the Venn diagram depicts how Pb can cycle through multiple environmental media prior to human exposure. The “air/soil/water” arrows illustrate Pb exposures to plants, animals, and/or humans via contact with Pb-containing media. The exposures are air-related if Pb passed through the air compartment. When animals consume plant material or water exposed to Pb that has at some point passed through the air compartment, and when human diet includes animals, plants or drinking water exposed to Pb that has passed through the air compartment, these are also considered air-related Pb exposures. As a result of the multitude of possible air-related exposure scenarios and the related difficulty of constructing Pb exposure histories, most studies of Pb exposure through air, water, and soil can be informative to this review. Figure 3-1 also illustrates other exposures, such as occupational exposures, contact with consumer goods in which Pb has been used, or ingestion of Pb in drinking water conveyed through Pb pipes. Most Pb biomarker studies do not indicate speciation or isotopic signature, and so exposures that are not related to Pb in ambient air are also reviewed in this section because they can contribute to Pb body burden. Many of the studies presented in the subsequent material focus on observations of Pb exposure via one medium: air, water, soil and dust, diet, or occupation.



Note: The Venn diagram is used to illustrate the passage of Pb through multiple environmental media compartments through which exposure can occur.

Figure 3-1 Conceptual model of multimedia Pb exposure.

The relative importance of different sources or pathways of potential exposure to Pb in the environment is often difficult to discern. Individual factors such as home environment, location, and risk factors (described in more detail in Chapter 5 and Chapter 6) may influence exposures. The National Human Exposure Assessment Survey (NHEXAS) study sampled Pb, as well as other pollutants and VOCs, in multiple exposure media from subjects across six states in EPA Region 5 (Illinois, Indiana, Michigan, Minnesota, Ohio, and Wisconsin) (Clayton et al., 1999) as well as in Arizona (O'Rourke et al., 1999) and Maryland (Egeghy et al., 2005). Results from NHEXAS indicate that personal exposure concentrations of Pb are higher than indoor or outdoor concentrations of Pb, perhaps suggesting a personal cloud effect; see Table 3-1. Pb levels in windowsill dust were higher than Pb levels in surface dust collected from other surfaces. Clayton et al. (1999) suggested that higher windowsill levels could be attributed to the presence of Pb-based paint and/or to accumulation of infiltrated outdoor Pb-bearing PM. Pb levels in food were higher than in beverages, and Pb levels in standing tap water (also referred to as “first flush” or “first draw”) were higher than Pb levels obtained after allowing water to run for three minutes to flush out pipes. Layton and Beamer (2009) estimated that 34-66% of Pb in floor dust was tracked in from outdoors and originated as

ambient air Pb, based on 1992 levels in Sacramento; in 1992, phase-out of Pb usage in gasoline was near complete, but industrial emissions were still higher than current levels; see Section 2.2.

Table 3-1 Estimates of Pb measurements for EPA Region 5 from the NHEXAS study.

Medium[a]	N	Percentage above LOD[b] (CLs)[c]	Mean (CLs)[c]	50th (CLs)[c]	90th (CLs)[c]
Personal air (ng/m^3)[d]	167	81.6 (71.3; 92.0)	26.83 (17.60; 36.06)	13.01 (11.13; 18.13)	57.20 (31.18; 85.10)
Indoor air (ng/m^3)[d]	213	49.8 (37.2; 62.3)	14.37 (8.76; 19.98)	6.61 (4.99; 8.15)	18.50 (12.69; 30.31)
Outdoor air (ng/m^3)[d]	87	73.8 (56.3; 91.3)	11.32 (8.16; 14.47)	8.50 (7.14; 10.35)	20.36 (12.60; 34.91)
Surface dust (ng/cm^2)	245	92.1 (87.4; 96.8)	514.43 (-336.6; 1365.5)	5.96 (3.37; 10.94)	84.23 (26.52; 442.63)
Surface dust (mg/kg)	244	92.1 (87.4; 96.8)	463.09 (188.15; 738.04)	120.12 (83.85; 160.59)	698.92 (411.84; 1,062.8)
Window sill dust (ng/cm^2)	239	95.8 (92.5; 99.0)	1,822.6 (481.49; 3,163.6)	16.76 (10.44; 39.41)	439.73 (106.34; 4,436.2)
Window sill dust (mg/kg)	239	95.8 (92.5; 99.0)	954.07 (506.70; 1,401.4)	191.43 (140.48; 256.65)	1,842.8 (1,151.3; 2,782.5)
Standing tap water (µg/L)	444	98.8 (97.6; 100.0)	3.92 (3.06; 4.79)	1.92 (1.49; 2.74)	9.34 (7.87; 12.35)
Flushed tap water (µg/L)	443	78.7 (70.7; 86.7)	0.84 (0.60; 1.07)	0.33 (0.23; 0.49)	1.85 (1.21; 3.04)
Solid food (µg/kg)	159	100.0 (100.0; 100.0)	10.47 (6.87; 14.07)	6.88 (6.44; 8.04)	14.88 (10.78; 19.08)

Table 3-1 (Continued): Estimates of Pb measurements for EPA Region 5 from the NHEXAS study.

Medium[a]	N	Percentage above LOD[b] (CLs)[c]	Mean (CLs)[c]	50th (CLs)[c]	90th (CLs)[c]
Beverages (µg/kg)	160	91.5 (85.2; 97.8)	1.42 (1.13; 1.72)	0.99 (0.84; 1.21)	2.47 (2.06; 3.59)
Food + Beverages (µg/kg)	156	100.0 (100.0; 100.0)	4.48 (2.94; 6.02)	3.10 (2.66; 3.52)	6.37 (4.89; 8.00)
Food intake (µg/day)	159	100.0 (100.0; 100.0)	7.96 (4.25; 11.68)	4.56 (3.68; 5.36)	12.61 (9.27; 16.38)
Beverage intake (µg/day)	160	91.5 (85.2; 97.8)	2.15 (1.66; 2.64)	1.41 (1.18; 1.60)	4.45 (3.15; 5.65)
Food + Beverage intake (µg/day)	156	100.0 (100.0; 100.0)	10.20 (6.52; 13.89)	6.40 (5.21; 7.78)	16.05 (13.31; 18.85)
Blood (µg/dL)	165	94.2 (88.2; 100.0)	2.18 (1.78; 2.58)	1.61 (1.41; 2.17)	4.05 (3.24; 5.18)

Note: EPA Region 5 includes six states: Illinois, Indiana, Ohio, Michigan, Minnesota, and Wisconsin. Participants were enrolled using a stratified, four-stage probability sampling design, and submitted questionnaire and physical measurements data. Summary statistics (percentage above limit of detection (LOD), mean, median, 90th percentile) were computed using weighted sample data analysis. The estimates apply to the larger Region 5 target population (all non-institutionalized residents residing in households).

[a]Estimates for indoor air, outdoor air, dust media, and water media apply to the target population of Region 5 households; estimates for other media apply to the target population of Region 5 residents.

[b]Percentage of the target population of residents (or households) estimated to have Pb levels above limit of detection (LOD).

[c]The lower and upper bounds of the 95% confidence limits (CL) are provided.

[d]PM_{50}.

Source: Reprinted with permission of Nature Publishing Group, Clayton et al. (1999)

3.1.1.1 Particle Size Distributions for Airborne-Pb, Dust-Pb, and Soil-Pb

The size distribution of ingestible dust particles differs from the size distribution of inhalable ambient air Pb particles and therefore cannot be directly compared. The inhalability of airborne PM is a gradually decreasing function of particle size. Inhalability criteria established from experimental data, obtained at wind speeds of 1-8 meters/second, describe PM inhalability of 77% for particles <10 µm (d_{ae}, aerodynamic diameter). Inhalability of particles ranging in size from 40 to 100 µm d_{ae} is 50%; above 100 µm, inhalability data are lacking (Soderholm, 1989; ACGIH, 1985). The particles that are not inhaled may settle to surfaces, making them available for subsequent ingestion. The size distribution of soil and house dust particles tends to be much larger than airborne PM. Que Hee et al. (1985) and U.S. EPA (1990b) observed that 50% or more of the mass of house dust tends to be comprised of particles smaller than 150 µm. Gulson et al. (1995b) observed that the mode of the Pb house dust size distribution was in the 38-53 µm range; they did not report the overall house dust size distribution. Given the house dust Pb size distributions documented, dust Pb brought into homes with foot traffic

may be aerosolized but is likely to stay airborne for only a few seconds, since particles larger than $PM_{2.5}$ tend to settle from the air quickly; see Section 2.3.1.3 and Section 3.1.3.1. Siciliano et al. (2009) observed different size distributions for different types of soils: agricultural sites had median soil Pb of 34 µm, and brownfields had median soil Pb of 105 µm. These observations of larger particle sizes for soil and dust Pb support the notion that exposure to Pb in dusts and soils would occur by ingestion rather than inhalation following resuspension.

The main pathway for Pb ingestion by children is by hand to mouth contact (Lanphear et al., 1998). In a playground environment in London, U.K., Duggan et al. (1985) reported that hand to mouth transfer was effectively limited to particles smaller than 10 µm, even when the soil itself exhibited a much larger particle size distribution. More recently, Yamamoto et al. (2006) reported for a cohort of children in Kanagawa Prefecture, Japan (greater Tokyo area) that the mode of size distributions of particles adhering to children's hands was 39 ± 26 µm, with the upper tail ranging from 200-300 µm. Kissel et al. (1996) measured three size fractions of soil adhered to a hand via a hand press: ≤ 150 µm, 150-250 µm, and ≥ 250 µm and observed that, when soil was dry (<2% moisture content), 43%-69% of the soil was in the smallest fraction. When the moisture content was higher than 2%, 28-81% of the adhered soil was in larger than 250 µm. Percentage and mass adhered per area (mg/cm^2) depended on soil type, with wet sand and loamy sand adhering more to hands than sandy loam or silt loam. For dry soil, silt loam mass produced the largest adherence in terms of mass per area. Differences among the size distribution results may be related to differences in the soil type, soil moisture levels between the locations, and/or to differences between the analytical methods used to measure size distribution; Duggan et al. (1985) used optical microscopy of the dust wipes, while Yamamoto et al. (2006) used a laser scattering device measuring sampled particles suspended in an aqueous solution. Siciliano et al. (2009) regressed adhered average soil size on hands bulk soil particle size and found a log-log relationship with $\beta = 0.66$ using both brownfield and agricultural soils; the proportion of soil adhered depended on organic content.

Several studies have found that Pb is enriched in the smaller particle sizes of the soil and house dust. Davies and White (1981) observed that enrichment decreased linearly with increasing dust size bin, with dust particles smaller than 64 µm having a Pb concentration of 76.1 mg/kg and particles in the 1,000-2,000 µm size range having a Pb concentration of 16.4 mg/kg. Sheets and Bergquist (1999) also found that Pb content decreased with increasing particle size. More recently, Ljung et al. (2006) investigated childhood exposures to trace metals on playgrounds in Uppsala, Sweden and observed that the Pb content in soil in the <50 µm size fraction was 1.5 times higher than that in the >4 mm or

50-100 μm size fractions. Sheppard et al. (1995) measured enrichment in different types of soils (sand and clay) and found that enrichment was substantially higher in the sand.

Studies focusing on particle size distributions of house dust adhered to the hands are lacking. Ingestion of house dust has been reported to be the major source of Pb intake during early childhood (Lanphear et al., 2002). If a similar particle size distribution holds for household dust, then ingestion of indoor Pb of atmospheric origin could also be strongly dependent on dust particle size. Therefore, larger particles of atmospheric origin, which may not be considered relevant for exposure by inhalation exposure, are still relevant for Pb exposure by ingestion. However, no studies in the literature have presented information on the relative contributions of Pb from different PM size fractions to blood Pb concentrations.

It should be noted that different measurement techniques are used for different environmental media. For example, ambient air Pb-PM size distribution is measured by one of the non-FRM instruments such as a MOUDI, described in Section 2.4, and its measurement is subject to errors specific to the technique. Dust and soil size distribution are typically measured with graduated sieves, and errors associated with these methods occur more often in the smaller size fractions that are subject to agglomeration and clogging if the particle shape is nonspherical.

3.1.1.2 Estimating Pb Exposure in the Integrated Exposure Uptake Biokinetic (IEUBK) Model

Several studies have used a combination of measured values and default model values to represent exposures and determine their relative contributions to blood Pb. For example, Cornelis et al. (2006) used the Integrated Exposure Uptake Biokinetic model (IEUBK), described in detail in the 2006 Pb AQCD (U.S. EPA, 2006b) to model children's exposures to Pb emissions from a non-ferrous smelter in Hoboken, Belgium. In deriving the model input (annual averages) for ambient air Pb concentration, as well as soil and dust, they employed weighting based on children's time spent in different locations in the study area and air, soil and indoor dust measurements in those areas. In their results for the area of the smelter, the ingestion of dust and soil pathways accounted for more than 70% of the exposure, while the inhalation pathway accounted for less than 2%. Similarly, Carrizales et al. (2006) analyzed exposures to children living near a copper (Cu) smelter in San Luis Potosi, Mexico. They employed the IEUBK default options for assignment of Pb dust concentration as 70% of the soil Pb concentration, while air Pb concentration was assigned based on measurements by the Mexican government. Based on these assumptions, they attributed 87% of blood Pb to soil and dust exposure. These studies did

not estimate the air Pb contribution to the soil/dust Pb concentrations and consequently did not estimate the portion of the ingestion pathway that derives from ambient air Pb.

Appendix I of the 2007 Pb Risk Assessment (U.S. EPA, 2007f) provides estimates of the contribution of various pathways to the blood Pb of children simulated in several case studies. Simulations provided estimates of contributions from outdoor ambient air Pb by inhalation and by ingestion of indoor dust, including the fraction of indoor dust Pb associated with recent penetration of ambient air Pb into the residence. Although ambient air Pb may also contribute to Pb ingestion through other pathways (i.e., diet, soil), data and tools to support a simulation of the linkage between air Pb concentrations and concentrations in other media were limited. Accordingly, Pb concentrations pertaining to other pathways (e.g., diet, outdoor soil, the component of indoor dust Pb other than that derived from Pb recently in ambient air) were held constant across the different air quality scenarios simulated. Table 3-2 provides estimates for the General Urban Case Study in the 2007 Pb Risk Assessment (U.S. EPA, 2007f). The General Urban Case Study, unlike the various location-specific case studies, was not based on any specific urban location and reflected several simplifying assumptions including uniform ambient air Pb levels across the simulated, hypothetical study area and a uniform study population.

Table 3-2 Predicted concurrent blood Pb levels and source contributions for children in their seventh year of life.

Air Pb[a] (µg/m^3)	Median Blood Pb[b] (µg/dL)	Pathway Contribution (%)				
		Ingestion				Inhalation
				Indoor Dust		
		Diet[c]	Outdoor Soil/Dust	Other[d]	Pb recently in air[e]	Ambient Air
0.05	1.7 (5.7)[f]	32	44	11	12.6	0.1
0.14[g]	1.9 (6.5)	28	38	6	28.3	0.5
0.2	2.0 (6.9)	26	36	5	32.7	0.7
0.87[h]	2.1 (7.2)	25	33	4	37.2	0.9

[a]Concentrations are maximum calendar quarter averages of Pb in air with exception of 0.05 µg/m^3 which is a maximum monthly average

[b]Average of blood Pb concentrations at 75 and 81 months, assuming exposure concentrations were constant through 7 years of life

[c]Includes food and drinking water, the Pb concentrations for which were held constant across the different air quality scenarios, as were Pb concentrations for outdoor soil/dust.

[d]Includes indoor dust with Pb contributions from sources other than Pb recently in the air (e.g., indoor paint, outdoor soil/dust, and additional sources including historical air Pb)

[e]Includes contributions associated with outdoor ambient air Pb from ingestion of indoor dust predicted to be associated with outdoor ambient air Pb levels

[f]Values in parentheses are the 95th percentile blood Pb for a geometric standard deviation of 2.1

[g]Mean of the maximum quarterly average concentrations of Pb in TSP (for period 2003 to 2005) among monitor locations in urban areas having more than one million residents

[h]95th percentile of the maximum quarterly average concentration of Pb in TSP (for period 2003 to 2005) among monitor locations in urban areas having more than one million residents

Source: Based on General Urban Case Study (Hybrid Dust Model) in Appendix I, 2007 Pb Risk Assessment (U.S. EPA, 2007f).

3.1.2 Environmental Exposure Assessment Methodologies

A number of monitoring and modeling techniques have been employed for exposure assessment. These are detailed in either the 2006 Pb AQCD (U.S. EPA, 2006b) or in the subsequent Risk and Exposure Assessment performed as part of the same NAAQS review (U.S. EPA, 2007g). Some of these methods are briefly described here to provide a context for the exposure studies described in Section 3.1.3. Blood Pb sampling is described in detail in Section 3.3.2.

Data collection to assess Pb exposure pathways may involve air, soil, and dust samples. Methods used for digesting air Pb samples are described in Section 2.4, as are ambient air Pb monitoring techniques. Factors affecting collection of ambient air Pb samples are described in detail in Section 2.4. For the monitors in the FRM network, the primary role is compliance assessment. Accordingly, this network includes monitors in locations near sources of air Pb emissions which are expected to or have been shown to contribute to ambient air Pb concentrations in excess of the Pb NAAQS. In such locations, Pb may be

associated with relatively larger size particles, contributing to air Pb concentration gradients with distance from the source and greater deposition in the near-source locations. The FRM network also includes non-source-oriented monitors for which the main objective is to gather information on neighborhood-scale Pb concentrations that are typical in urban areas so to better understand ambient air-related Pb exposures for populations in these areas. This part of the Pb NAAQS network, was required to be operational as of December 27, 2011. These monitor locations are distributed across a broad geographic area, representing approximately 63 large urban areas which contain approximately half of the total U.S. population (based on recently published 2010 Census Bureau data). In lieu of more detailed analysis of population proximity for these newly established monitors, population counts were calculated near previously existing monitors for which data are presented in Section 2.5. For the monitors in that limited dataset, among the total population of 311,127,619 people in the 2010 Census (ESRI, 2011), 181,100 (0.06%) lived within 1 km of a source-oriented monitor, while 918,351 (0.30%) lived within 1 km of a non-source-oriented monitor.

Dust sampling has not changed drastically since it was first proposed by Sayre et al. (1974), in which a disposable paper towel was soaked in 20% denatured alcohol and 1:750 benzalkonium chloride and then used to wipe a 1 ft^2 sampling area in a systematic fashion. Que Hee et al. (1985) and Sterling et al. (1999) compared wipe testing with vacuum methods. Sampling efficiency for the first attempt varied between 53-76% with vacuum pump flow rate and tube type and was 52% for the wipe method for the Que Hee et al. (1985) study, with 100% efficiency after five consecutive samples were obtained. Sterling et al. (1999) observed that two of three vacuuming methods had significantly higher geometric mean collection (vacuum 1: 94.3 µg/ft^2; vacuum 2: 23.5 µg/ft^2) compared with dust wipes (5.6 µg/ft^2).

Models may also be used in exposure assessment. For example, two dispersion models, the American Meteorological Society/Environmental Protection Agency Regulatory Model (AERMOD), and Industrial Source Complex-Plume Rise Model Enhancements (ISC-PRIME) were employed to model dispersion of Pb emissions from specific industrial facilities (Cimorelli et al., 2005; Perry et al., 2005; EPRI, 1997), and to estimate ambient air Pb concentrations at some of the case studies included in the 2007 Risk and Exposure Assessment (U.S. EPA, 2007g). These models assume plume dispersion follows a Gaussian distribution from a point source. For the two point source case studies included in the 2007 risk assessment, the plume models were used to track emissions to ambient air near homes located within a few miles of emitting facilities. However, dispersion models can also be used to track long distance transport of Pb emissions, as performed by Krell and Roeckner (1988) to model the dispersion and deposition of Pb and Cd from European nations into the North Sea.

Several models estimate blood Pb levels resulting from estimated exposure to Pb in environmental media. These models, which are described in detail in the 2006 Pb AQCD (U.S. EPA, 2006b) include the IEUBK model, and the EPA *All Ages Lead Model* (AALM), which combines and expands the thorough exposure and absorption modules of the IEUBK model with the comprehensive biokinetic model of Leggett (1993). As of the writing of this assessment, the AALM is still in development.

The Stochastic Human Exposure and Dose (SHEDS) and NORMTOX models also are capable of modeling metals exposures through various routes including inhalation, ingestion, and dermal exposure (Loos M, 2010; Burke et al., 2002). Pb exposure modeling can also be accomplished using the Modeling Environment for Total Risk (MENTOR) framework, in which airborne Pb levels could be modeled using AQS, dispersion modeling, or chemical transport modeling, while human exposure is modeled with SHEDS or a similar exposure model (Georgopoulos and Lioy, 2006). Additionally, housing data and time-activity data from the Consolidated Human Activity Database (CHAD) are incorporated into MENTOR to develop refined estimates of Pb exposure and tissue burden. However, a literature search did not produce any Pb exposure studies using the SHEDS, NORMTOX, or MENTOR modeling systems. In general, these models take input for several environmental Pb exposure media including soil, dust, food and water, outdoor air, and indoor air. The models are designed to evaluate different exposure scenarios based on specification of particular conditions.

3.1.3 Exposure Studies

3.1.3.1 Airborne Pb Exposure

Limited personal exposure monitoring data for airborne Pb were available for the 2006 Pb AQCD (U.S. EPA, 2006b). As described above, the NHEXAS study showed personal air Pb concentrations to be significantly higher than indoor or outdoor air Pb concentrations (Clayton et al., 1999). Indoor air Pb concentration was moderately correlated with floor dust and residential yard soil Pb concentration (Rabinowitz et al., 1985). Egeghy et al. (2005) performed multivariate fixed effects analysis of the NHEXAS-Maryland data and found that Pb levels measured in indoor air were significantly associated with log-transformed outdoor air Pb levels, ambient temperature, number of hours in which windows were open, whether homes were built before 1950, and frequency of fireplace usage (Table 3-3).

Table 3-3 Estimates of fixed effects multivariate modeling of Pb levels measured during the NHEXAS-MD study.

	Pb in Indoor Air		Pb in Dust		Dermal Pb		Blood Pb	
Fixed Effect	**β[a]**	**p-value**	**β[a]**	**p-value**	**β[a]**	**p-value**	**β[a]**	**p-value**
Intercept	-0.50	0.0051	6.22	<0.0001	6.23	<0.0001	0.02	0.91
Outdoor Pb concentration[b]	0.51	<0.0001						
Average weekly temperature (°F)	0.01	0.046						
Open window periods (hr)	0.01	0.035	-0.03	0.0082				
House pets (yes)	-0.15	0.078						
Air filter use (yes)	-0.28	0.087					-0.12	0.088
Home age (<1950)	0.25	0.025	0.96	0.029				
Fireplace (frequency of use)	0.11	0.045	0.46	0.0054				
Pb concentration in soil[b]			0.27	0.037				
Interior Pb paint chipping/peeling (yes)			0.43	0.091				
Cement at primary entryway (yes)			1.97	0.0064				
Indoor pesticide usage last 6 mo (yes)			-0.78	0.0003				
Electrostatic air filter usage (yes)			-0.91	0.062				
Sex of participants (male)					0.41	0.0012	0.43	<0.0001
Ethnic minority participants (yes)					0.41	0.0063		
Washing hands after lawn mowing (no)					1.04	0.0010		
Gasoline power- equipment usage (yes)					0.61	0.0072		
Bathing or showering activities (yes)					-0.43	0.019		
Dust level indoors (scale: 1-3)					0.22	0.019		
Residing near commercial areas (yes)					0.32	0.0087		
Age of participants (yr)							0.02	<0.0001
Number cigarettes smoked (count)							0.03	<0.0001
Burning wood or trash (days)							0.58	0.0099
Showering frequency (avg # days)							-0.29	0.0064
Work outside home (yes)							-0.26	<0.0001
Health status (good)							0.23	0.0009
Adherence to high fiber diet (yes)							-0.15	0.040
Gas or charcoal grill usage (yes)							-0.17	0.0002

[a]Estimates of fixed effects in final multiple regression analysis models for Pb in the Maryland investigation data in the National Human Exposure Assessment Survey (NHEXAS-MD).

[b]Log transform.

Source: Reprinted with permission of Nature Publishing Group, Egeghy et al. (2005).

Some recent studies have shown that the ratio of indoor to outdoor Pb-PM varies from site to site depending on factors including infiltration, indoor and outdoor Pb sources, and meteorology. Adgate et al. (2007) measured the concentrations of several trace elements in personal, indoor, and outdoor air samples of $PM_{2.5}$ and found that average personal Pb-$PM_{2.5}$ concentration was roughly three times higher than outdoor air Pb-$PM_{2.5}$ concentration and two times higher than indoor Pb-$PM_{2.5}$ concentration (Table 3-4). Another study of indoor and outdoor air concentrations of Pb was carried out by Molnar et al. (2007). $PM_{2.5}$ trace element concentrations were determined in homes, preschools and schools in Stockholm, Sweden. In all sampled locations, Pb-$PM_{2.5}$ concentrations were higher in the outdoor environment than in the proximal indoor environment. The indoor/outdoor ratios for Pb-$PM_{2.5}$ suggest an outdoor Pb-$PM_{2.5}$ net infiltration of ~0.6 for these buildings. Outdoor air Pb concentrations did not differ between the central and more rural locations. Indoor air Pb concentrations were higher in spring than in winter, which the authors attributed to greater resuspension of elements that had accumulated in road dust over the winter period and increased road wear on days with dry surfaces. Pekey et al. (2010) measured indoor and outdoor trace element composition of $PM_{2.5}$ and PM_{10} in Kocaeli, an industrial region of Turkey, and found that average airborne Pb concentrations were higher outdoors than indoors for both $PM_{2.5}$ and PM_{10} during summer and for PM_{10} during winter, but that indoor Pb concentration was higher than outdoor Pb concentration for $PM_{2.5}$ during winter. The indoor-to-outdoor ratio of airborne Pb varied by environment; it tended to be less than one, but the ratio varied from one microenvironment to another. In a pilot study in Windsor, Ontario, Rasmussen et al. (2007) observed that the concentration of Pb in $PM_{2.5}$ from a personal exposure sample was roughly 40% higher than the concentration of Pb in outdoor $PM_{2.5}$ and 150% higher than Pb in indoor $PM_{2.5}$. The three studies that included personal samples recorded measurements that were consistently higher than indoor or outdoor levels, and outdoor concentrations were higher than indoor concentrations.

Domestic wood burning is a potential source of Pb compounds (Section 2.2.2.5). Alves et al. (2011) measured trace metals in woodstove and fireplace emissions and found that $PM_{2.5}$ contained Pb, with concentrations from wood burning ranging from 3.3-12.2 µg/g and 2.89-30.3 for woodstoves and fireplaces, respectively. When burning briquettes, the $PM_{2.5}$ measurements showed Pb enrichment above all other metal elements other than potassium (woodstove: 1361 µg/g; fireplace: 616 µg/g). Molnar et al. (2005) measured trace element concentration in indoor and personal exposure $PM_{2.5}$ samples for homes in which wood is burned and in a reference group where no wood burning occurs in the home. For both indoor and personal samples, Molnar et al. (2005) observed that Pb concentrations were higher for the wood burning group and nearly statistically significant for the personal exposure samples (indoor concentration: 6.0 µg/m^3 versus 4.3 µg/m^3, $p = 0.26$; personal exposure: 4.6 µg/m^3 versus 3.0 µg/m^3, $p = 0.06$).

Indoor activity has been associated with resuspension of settled dust, which could cause airborne contact with particle-bound Pb. Qian et al. (2008) estimated a PM_{10} resuspension rate of $1.4x10^{-4}$/hr for one person walking across a carpeted floor. Measurements of submicron particles illustrated a roughly two-fold increase of airborne particle concentration for particles smaller than 1.8 µm for activity versus low activity periods, with maximum concentrations reaching 4-11 times the maximum value during low activity periods. For PM_{10}, average concentration was 2.5 times higher than background levels during activity periods, while peak concentration was 4.5 times higher. Qian and Ferro (2008) observed that resuspension rates depend on particle size, floor material, and ventilation position. Increases in walking speed and weight of the walker did not consistently produce increases in resuspension. 5-10 µm particles produced a higher resuspension rate compared with smaller particles. Newly carpeted areas produced significantly higher resuspension rates than vinyl floors. Zhang et al. (2008) modeled and conducted experiments of particle dispersion from walking and observed that human activity did affect resuspension. They found that larger particles were more readily detached from the carpet by walking motion, but that smaller particles are more easily resuspended once detached. Hunt and Johnson (2012) studied the duration and spatial extent of resuspension of 0.3-5.0 µm particles following walking by a soiled shoe. 0.3-0.5 µm particle concentration remained increased over a time period of 23 min, while 1-5 µm particles declined in concentration over the same time period. Experiments and computational fluid dynamics simulations by Eisner et al. (2010) for a mechanical foot moving on carpeting suggested that the rotating motion of the moving foot on the carpet induced rotating air movement beneath the foot that re-entrained the particles.

Several of the studies can be used to develop an understanding of how personal exposure to PM-bound Pb varies with other exposures. Molnar et al. (2007) reported Spearman correlations of Pb with $PM_{2.5}$ and NO_2 in three outdoor microenvironments (residence, school, and preschool) and found that Pb and other trace metals were generally well correlated with $PM_{2.5}$ ($r = 0.72$ to 0.85), but Pb was only statistically significantly correlated with NO_2 in one of the three outdoor microenvironments ($r = 0.24$ [residence and school], versus 0.75 [preschool]). Pb was attributed by Molnar et al. (2007) to long range transport. Table 3-4 illustrates that measured Pb concentrations (summarized in Table 2-29 of the Chapter 2 Appendix [Section 2.8]) are typically well below the level of the NAAQS. The higher personal air concentrations occurred in a heavily industrialized area of Kocaeli, Turkey with an incinerator and several industrial facilities including metal processing, cement, petroleum refining, and agriculture processing. Otherwise, concentrations were all between 0.002 and 0.006 $\mu g/m^3$. The proportion of Pb compared with other trace metals varied with location and component. It was typically several times lower than S as well as crustal elements such as Ca^{2+} and Fe. In the industrial area of Kocaeli, Pb comprised a greater proportion of the PM compared with other areas.

Table 3-4 Comparison of personal, indoor, and outdoor Pb-PM measurements from several studies.

Study	Location	Pb Metric	Sampling Period	Personal Pb	Indoor Pb	Outdoor Pb
Clayton et al. (1999)	IL, IN, MI, MN, OH, WI	Med. Pb-PM_{50} (ng/m^3)	July, 1995-May, 1997	13	6.6	8.5
Adgate et al. (2007)	Minneapolis-St. Paul, MN	Avg. Pb-$PM_{2.5}$ (ng/m^3)	Spring, Summer, Fall, 1999	6.2	3.4	2.0
Molnar et al. (2007)	Stockholm, Sweden	Avg. Pb-$PM_{2.5}$ (ng/m^3)	December, 2003-July, 2004		Homes: 3.4 Schools: 2.5 Preschools: 1.8	Homes: 4.5 Schools: 4.6 Preschools: 2.6
Tovalin-Ahumada et al. (2007)	Mexico City, Mexico	Med. Pb-$PM_{2.5}$ (ng/m^3)	April-May, 2002		26	56
	Puebla, Mexico	Med. Pb-$PM_{2.5}$ (ng/m^3)	April-May, 2002		4	4
Pekey et al. (2010)	Kocaeli, Turkey	Avg. Pb-$PM_{2.5}$ (ng/m^3)	May-June, 2006, December, 2006-January 2007		Summer: 34 Winter: 85	Summer: 47 Winter: 72
		Avg. Pb-PM_{10} (ng/m^3)	May-June, 2006, December, 2006-January 2007		Summer: 57 Winter: 125	Summer: 78 Winter: 159
Rasmussen et al. (2007)	Windsor, Ontario, Canada	Med. Pb-$PM_{2.5}$ (mg/kg)	April, 2004	311	124	221

3.1.3.2 Exposure to Pb in Soil and Dust

The 2006 Pb AQCD (U.S. EPA, 2006b) lists indoor Pb dust infiltrated from outdoors as a potential source of exposure to Pb soil and dust. Thus, outdoor soil Pb may present an inhalation exposure if resuspended indoors or an ingestion exposure during hand-to-mouth contact. A detailed description of studies of outdoor soil Pb concentration is provided in Section 2.6.1. Indoor measurements can reflect infiltrated Pb as well as Pb dust derived from debrided paint, consumer products, or soil that has been transported into the home via foot traffic. Table 3-5 presents indoor dust Pb concentrations for 2006-2011 observational studies in which indoor dust Pb was measured.

Table 3-5 Measurements of indoor dust Pb concentration from 2006-2011 studies.

Reference	Study Location	Metric (units)	Sample Site	Indoor Pb Concentration
Caravanos et al. (2006b)	New York City, New York	Weekly dust loading (µg/m^2)	Glass plate next to open window of academic building	Median: 52
Khoder et al. (2010)	Giza, Egypt (extensive leaded gasoline use; industrial area)	Weekly dust loading (µg/m^2)	Glass plate in second-floor living room of apartments	Median: 408
Brattin and Griffin (2011)	Eureka, Utah near Eureka Mills Superfund Site	Dust concentration (mg/kg)	Indoor home site (not specified)	160-2000
	Denver, CO, near VBI70 Superfund Site		Indoor home site (not specified)	11-660
	East Helena, MT, near East Helena Superfund Site		Indoor home site (not specified)	68-1000
Yu et al. (2006)	Syracuse, New York	Dust concentration range (mg/kg)	Floor	Range: 209-1770
Turner and Simmonds (2006)	Birmingham, Plymouth, and 2 rural sites, U.K.	Dust concentration (mg/kg)	Floor	Median: 178
Gaitens et al. (2009)	U.S. (nationwide)	Dust loading (µg/m^2)	Smooth floor	Median: 1.7 Avg.: 4.4
			Rough floor	Median: 5.6 Avg.: 16
			Smooth windowsill	Median: 2.5 Avg.: 190
			Rough windowsill	Median: 55 Avg.: 480
Wilson et al. (2007)	Milwaukee, Wisconsin	Dust concentration (µg/m^2)	Central perimeter	Avg.: 107
			Entry	Avg.: 140
			Window	Avg.: 151
Zota et al. (2011)	Ottawa County, Oklahoma (area surrounding the Tar Creek Superfund Site)	Dust concentration (mg/kg)	Indoor (site not specified)	Avg.: 109 Median: 63 Max.: 881
Spalinger et al. (2007)	Rural towns, Idaho	Dust concentration (mg/kg)	Vacuum	Median: 120 Max: 830
			Floor	Median: 95 Max: 1,300
	Bunker Hill, Idaho Superfund site	Dust concentration (mg/kg)	Vacuum	Median: 470 Max: 2,000
			Floor	Median: 290 Max: 4,600

Several studies suggested the infiltration of Pb dust into buildings. For example, Caravanos et al. (2006b) collected dust on glass plates at an interior location near an open window, a sheltered exterior location, and an open exterior location for a two-year period in Manhattan, NY. Median weekly dust loading was reported to be 52 µg/m^2 for the indoor site, 153 µg/m^2 for the unsheltered outdoor site, and 347 µg/m^2 for the sheltered outdoor site. This paper demonstrated the likely role of outdoor Pb in influencing indoor dust Pb loading and indicated that under quiescent conditions (e.g., no cleaning) near an open second-story window, the indoor dust Pb level might exceed EPA's hazard level for interior floor dust of 430 µg/m^2 (40 µg/ft^2). Khoder et al. (2010) used the same methodology to study Pb dust deposition in residential households in the town of Giza, Egypt, located between two industrial areas and where leaded gasoline is still in use; the investigators reported a median weekly deposition rate of 408 µg/m^2 and an exterior median deposition rate of 2,600 µg/m^2. In the latter study, Pb deposition rate correlated with total dust deposition rate (R=0.92), Cd deposition rate (R=0.95), and Ni deposition rate (R=0.90). Statistically significant differences in Pb deposition rates were observed between old and new homes ($p < 0.01$) in the Khoder et al. (2010) study, although the only quantitative information provided regarding home age stated that the oldest home was 22 years old when the study was performed in 2007. Khoder et al. (2010) found no statistically significant difference between Pb loadings when segregating the data by proximity to roadways. Recently, Brattin and Griffin (2011) performed linear regressions of dust Pb on soil Pb based on data collected previously for outdoor soil Pb and indoor dust Pb near mining and/or smelting Superfund sites in Utah, Colorado, and Montana (U.S. EPA, 2005f; SRC, 2002; U.S. EPA, 2001). They observed that the dust Pb concentration was 4-35% of outdoor soil Pb. Excluding outliers on the regression, dust Pb concentration ranged from 160-2,000 mg/kg, 11-660 mg/kg, and 68-1,000 mg/kg at three sites.

Correlations between indoor and outdoor Pb content in dust can be partially explained with speciation. Beauchemin et al. (2011) used XANES to speciate in-home paint samples to assess the contributions of indoor paint and outdoor material to indoor dust Pb concentrations. In indoor dust samples of particles <150 µm in size, Pb oxide, Pb sulfate, and Pb carbonate were measured. These materials commonly were used in white paint. In the size fraction of particles <36 µm, half of the measured Pb was associated with Fe-oxyhydroxides such as ferrihydrite and goethite and presumably adsorbed onto these species. This finding suggested that a mix of indoor and outdoor sources may affect the composition of dust in the smaller size fraction in houses with leaded paint.

Residual Pb dust contamination following cleaning activities has been documented. For instance, Hunt et al. (2008) estimated Pb deposition and concentration from experiments in which Herculaneum, MO yard soil samples that had been dried, ground, and sieved

were tracked onto a tile test surface and then repeatedly cleaned until visual inspection of the tiles uncovered no surface discoloration. Cleaning resulted in a 5-6 fold decrease in residual Pb, with 7,100 µg/m^2 measured after multiple walks across the sample floor prior to cleaning. Yu et al. (2006) analyzed dust samples from 50 homes in northern New Jersey (typically of older housing stock, although the study does not specify housing age). The investigators found that total Pb concentration in carpet dust ranged from 209 to 1,770 mg/kg dust. Wilson et al. (2007) studied Pb dust samples from homes in Milwaukee, WI, and in resident children with and without elevated blood Pb ≥ 10 µg/dL. They found that Pb dust samples obtained from the floor were always significantly higher in residences of children with elevated blood Pb, with the exception of samples from the bathroom floor. Windowsill dust was not significantly higher in residences of children with elevated blood Pb. Residual Pb dust in homes is a potential exposure source for small children who use touch to explore their environments.

Pb dust on floors, windowsills, and other accessible surfaces is related to several demographic, socioeconomic, and housing conditions. Gaitens et al. (2009) used NHANES data from 1999 through 2004 to examine Pb in dust in homes of children ages 12-60 months. Floor Pb dust loading value was modeled against several survey covariates and was significantly associated with several covariates but with mixed sign ($p < 0.05$). Floor Pb dust was positively associated with windowsill Pb dust loading, being of non-Hispanic black race/ethnicity, and presence of smokers in the home. Floor Pb dust was negatively associated with presence of carpeting, poverty-to-income ratio, and living in a home built after 1950. It was nearly significantly and positively associated ($p = 0.056$) with renovations made to pre-1950 homes. Windowsill Pb dust level was also significantly associated ($p < 0.05$) with several covariates. It was positively associated with being of non-Hispanic black race/ethnicity, negatively associated with living in a home built after 1950, positively associated with not smooth and cleanable window surface condition, positively associated with presence of smokers in the home, and positively associated with deterioration of indoor paint. It was nearly statistically significantly and positively associated ($p = 0.076$) with deterioration of outdoor paint when homes were built prior to 1950. Dust Pb loading was found by Egeghy et al. (2005) to be significantly and positively associated with the log-transform of soil Pb concentration, cement content in the home entryway, frequency of fireplace usage, and homes built before 1950. Dust Pb loading was significantly and negatively associated with indoor pesticide use and number of hours in which windows were open (Table 3-3).

Building demolition and renovation activities can create dust from interior and exterior paints with Pb content. Mielke and Gonzales (2008) measured Pb content in paint chips from paint applied prior to 1992 and found that median Pb levels were 420 mg/kg for interior paint and 77,000 mg/kg for exterior paint. Maximum levels were 63,000 mg/kg

and 120,000 mg/kg for interior and exterior paint, respectively. Mielke et al. (2001) compared dust samples from two New Orleans houses that were prepared for painting. One home was power sanded without any confinement or control of removed material, while the other was hand-scraped with containment and collection of paint chips. Immediately after sanding, Pb dust samples ranged from <32 to 300,000 μg/m^2 at the sanded house. Pb dust samples from the scraped house ranged from 11 to 30,000 μg/m^2.

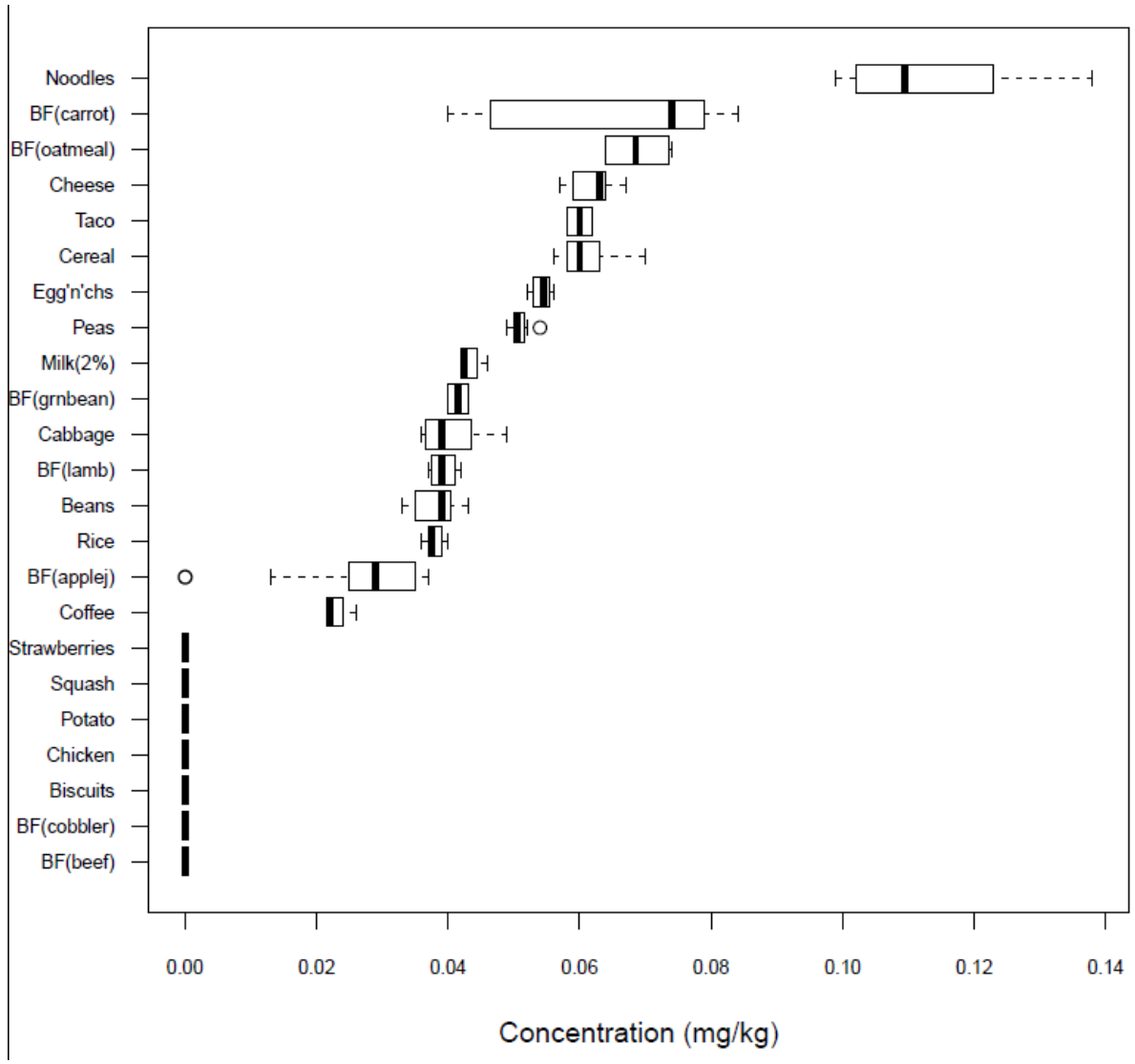
Dust Pb concentrations have also been reported for homes in the vicinity of historic and active metals mining and smelting sources. As described in Section 2.6.1, soil Pb has been found to be elevated near source of ambient air Pb. Near an active smelter in Port Pirie, Australia, median hand dust Pb loadings increased with age among a cohort of fourteen children followed over age 0-36 months (2-5 months: 54 μg/m^2, >15 months: 336 μg/m^2) (Simon et al., 2007). Zota et al. (2011) studied Pb dust and indoor Pb-$PM_{2.5}$ concentration in Ottawa County, OK near the Tar Creek Superfund Site, in which a metals mine had closed. Statistically significant correlations among outdoor soil Pb concentration, indoor dust Pb concentration, indoor dust Pb loading, and indoor air Pb-$PM_{2.5}$ concentrations were observed (r = 0.25-0.65), with an average dust Pb concentration of 109 mg/kg, dust Pb loading of 54 μg/m^2, soil Pb concentration of 201 mg/kg, and indoor Pb-$PM_{2.5}$ concentration of 1 ng/m^3. House dust Pb concentrations were found to increase significantly with residential proximity to two chat (i.e., dry mining waste) sources and to decrease with distance to the street and presence of central air conditioning. Spalinger et al. (2007) measured Pb in dust in homes in a 34 km^2 area surrounding a designated Superfund site where a Pb and Zn smelter formerly operated at Bunker Hill, ID. During spring of 1999, vacuum and floor mat samples were taken from homes in three towns within the 34 km^2 area and five “background” towns further from the Superfund site. For the background towns, Pb concentration in vacuum dust had a median of 120 mg/kg, and Pb concentration in floor dust had a median of 95 mg/kg. The median Pb dust loading rate was measured to be 40 μg/m^2 per day. In contrast, Pb in vacuum dust and floor mats for the towns contained within the Bunker Hill Superfund site had a median Pb concentration of 470 mg/kg and 290 mg/kg, respectively. The median Pb loading rate for indoor dust in houses in these towns was 300 μg/m^2 per day. These results suggest that those living in close proximity to large Pb and Zn smelters or mines that are now Superfund sites are at much greater risk of exposure to Pb dust compared to the general population.

Pb exposure has been reported on children’s playgrounds. Mielke et al. (2011a) reported median soil Pb concentration of 558 mg/kg on playground soils at eleven New Orleans daycare or community centers. Following remediation efforts to cover playground soil with clean soil, median concentration dropped to 4.1 mg/kg. Duggan et al. (1985) reported on the concentration and size distribution of wipe samples on the hands of 368

pre-school children from eleven schools in London, U.K.. Hand Pb residue (PbH) values were modeled as linear ($p < 0.05$) and power functions ($p < 0.001$) of Pb dust; linear slope was 0.0064 µg hand Pb residue per mg/kg Pb dust. Given that the Duggan et al. (1985) study was performed when Pb additives were used in gasoline, dust Pb concentration values are not reported here. As described in Section 3.1.1.1, exposure to Pb in soil and dust may be related to size distribution of the soil or dust particles, with higher Pb enrichment in the smaller particles.

3.1.3.3 Dietary Pb Exposure

This subsection covers several dietary Pb exposures from a diverse set of sources. Included among those are drinking water, fish and meat, agriculture, urban gardening, dietary supplements, tobacco, cultural food sources, and breastfeeding. The breadth of dietary Pb exposures is illustrated in Figure 3-2, which illustrates the data obtained in the 2008 FDA Total Diet Study market basket survey (FDA, 2008). Among the highest Pb concentrations were those for noodles, baby food carrots, baby food oatmeal, Swiss cheese, beef tacos from a Mexican restaurant, and fruit-flavored cereal. Possible sources of Pb in food samples include introduction during processing or preparation with drinking water contaminated with Pb, deposition of Pb onto raw materials for each food, and Pb exposure in livestock that produce dairy or meat ingredients. Manton et al. (2005) used Pb isotope ratios to estimate sources of dietary Pb among a cohort of mothers and children from Omaha, NE using a combination of food samples, hand wipes, house dust wipes, and aerosol samples collected between 1990 and 1997. Drinking water Pb was not included in this study. The authors cited results from Egan et al. (2002) that imported vegetables contributed 55% of Pb dietary intake for infants, 30% for 2-6 year old children, and 20% for 25-30 year old women. Imported candy contributed 10% of Pb dietary intake for 2-6 year old children and 9% for 25-30 year old women. Isotopic data from Manton et al. (2005) suggested that, with the exception of children age 0-12 months, house dust is a large contributor to dietary Pb. The pattern of certain Pb-isotope ratios observed in the diet of children 0-12 months are suggested to derive from Ca salts in limestone that may have been used in dietary supplements in baby formula. The contribution of ambient air Pb to dietary Pb samples was not statistically significant for this urban exposure study.



Note: from the 2008 FDA Total Diet Study. "BF" denotes baby food.
Source Data: (FDA, 2008)

Figure 3-2 Market basket survey results for Pb concentration in foods.

Drinking Water

Pb concentrations in drinking water vary substantially. For example, Shotyk and Krachler (2009) measured the Pb concentration in tap water, commercially bottled tap water and bottled natural water. They found that, in many cases, tap water contained less Pb than bottled water. Excluding bottled water in glass containers because Pb can be leached from the glass, the median Pb concentration in the bottled water samples was 8.5 ng/L (range $\leq$ 1 to 761 ng/L). Pb in drinking water supplies can derive from atmospheric deposition onto surface waters, runoff of atmospheric deposition as described in Section 2.3, or via corrosion of Pb in the distribution network exacerbated by contact with acidic disinfection byproducts, as described in the following paragraphs.

It is now recognized that environmental nanoparticles (NPs) (~1-100 nm) can play a key role in determining the chemical characteristics of treated drinking water as well as natural waters (Wigginton et al., 2007). An important question is whether or not NPs from source waters affect the quality of drinking water. For example, if Fe-oxide NPs are not removed during the flocculation/coagulation stage of the treatment process, they may become effective transporters of contaminants such as Pb, particularly if these contaminants are leached from piping in the distribution system.

Corrosion byproducts can influence Pb concentrations in drinking water. Schock et al. (2008) characterized Pb pipe scales from 91 pipes made available from 26 different municipal water systems from across the northern U.S. They found a wide range of elements including Cu, Zn and V as well as Al, Fe and Mn. Interestingly, V was present at nearly one percent levels in pipes from many geographically diverse systems. In a separate study, Gerke et al. (2009) identified the corrosion product, vanadinite ($Pb_5(VO_4)_3Cl$) in Pb pipe corrosion byproducts collected from 15 Pb or Pb-lined pipes representing 8 different municipal drinking water distribution systems in the Northeastern and Midwest regions of the U.S. Vanadinite was most frequently found in the surface layers of the corrosion products. The vanadate ion, VO_4^{3-}, essentially replaces the phosphate ion in pyromorphite and hydroxyapatite structures. It is not known whether the application of orthophosphate as a corrosion inhibitor would destabilize vanadinite, but this substitution would have implications for V release into drinking water. The stability of vanadinite in the presence of monochloramine is also not known, and its stability might have implications for both Pb and V release into drinking water.

In recent years, drinking water treatment plants in many municipalities have switched from using chlorine to other disinfecting agents because their disinfection byproducts may be less carcinogenic. However, chloramines are more acidic than chlorine and can increase Pb solubility (Raab et al., 1991) and increase Pb concentrations in tap water. For example, after observing elevated Pb concentrations in drinking water samples, Kim and

Herrera (2010) observed Pb oxide corrosion scales occurring after using acidic alum as a disinfection agent. Edwards and Dudi (2004) observed a red-brown particle-bound Pb in Washington, D.C. water that could be confused with innocuous Fe. The source of the particle-bound Pb was not known but was thought to originate from the source water. The high Pb concentrations were attributed to leaching of Pb from Pb-bearing pipes promoted by breakdown products of disinfection agents (Edwards and Dudi, 2004). Maas et al. (2007) tested the effect of fluoridation and chlorine-based (chlorine and chloramines) disinfection agents on Pb leaching from plumbing soldered with Pb. When using chlorine disinfection agents alone, the Pb concentration in water samples doubled during the first week of application (from 100 to 200 ppb) but then decreased over time. When adding fluorosilicic acid and ammonia, the Pb concentration spiked to 900 ppb and increased further over time. However, Macek et al. (2006) regressed blood Pb among children ages 1-16 years on fluoride treatment, adjusted for several demographic and socioeconomic factors, and found no association when all data were combined into one model; when stratifying by housing age, Macek et al. (2006) found statistically significant odds ratios for those living in housing built before 1946 or for housing age unknown. Similarly, Lasheen et al. (2008) observed Pb leaching from pipes in Egypt when exposed to an acid of pH = 6. Exposure to basic solutions actually resulted in reduction of Pb concentration in the drinking water. Leaching of Pb from pipes following disinfection with acidic agents can lead to increased Pb exposure; Miranda et al. (2007b) observed a statistically significant association between blood Pb levels among children living in Wayne County, NC and use of chloramines ($p < 0.001$) in a log-linear model, although the study did not control for the presence of Pb paint in the dwellings, so it is difficult to distinguish the influence of Pb pipes from Pb in paint on blood Pb levels.

Several chemical mechanisms may contribute to release of Pb during use of chloramine disinfection agents. Edwards and Dudi (2004) hypothesized that Pb leaching occurs when chloramines cause the breakdown of brass alloys and solder containing Pb. After observing that nitrification also leads to increased Pb concentrations in water, they also proposed that chloramines may trigger nitrification and hence cause decreasing pH, alkalinity, and dissolved oxygen that leads to corrosion after observing that nitrification also leads to increased Pb concentrations in water. However, Zhang et al. (2009b) found no evidence that nitrification brought about significant leaching of Pb from Pb pipes. Lytle et al. (2009) suggested that a lack of increased Pb(II) concentrations in drinking water following a change from free chlorine to chloramine disinfection is attributed to the formation of the Pb(II) mineral hydroxypyromorphite ($Pb_5(PO_4)_3OH$) instead of Pb(IV) oxide. Xie et al. (2010) further investigated the mechanisms by which Pb(II) release is affected by chloramines. Two opposing mechanisms were proposed: $Pb(IV)O_2$ reduction by an intermediate species from decomposition of monochloramine; and increasing redox potential which decreases the thermodynamic driving force for reduction. They suggest

that the contact time of monochloramine with PbO_2 and the Cl_2:N ratio in monochloramine formation will determine which mechanism is more important. Free chlorine can control Pb concentrations from dissolution under flowing conditions but for long stagnation periods, Pb concentrations can exceed the action level: 4-10 days were required for Pb concentrations to exceed 15 µg/L (for relatively high loadings of PbO_2, of 1 g/L). Thus, under less extreme conditions, it was concluded that chloramination was unlikely to have a major effect on the release of Pb into drinking water.

Agriculture

The 2006 Pb AQCD (2006b) states that surface deposition "represents a significant contribution to the total Pb in and on the plant", while uptake through a plant's roots can also contribute to a plant's Pb concentration. Consequently, Pb content in plants may contribute to human dietary exposure. Uptake of Pb by plants growing in contaminated soil has been repeatedly demonstrated in some species during controlled potted plant experiments (Del Río-Celestino et al., 2006). In this study, most species retained Pb in the roots with little mobilization to the shoots of the plants. However, certain species of grasses were able to mobilize Pb from the roots to the shoots of the plant; these specific species could lead to human exposures through consumption of grazing animals. Lima et al. (2009) conducted similar greenhouse experiments with several vegetable crops grown in soil contaminated by Pb-containing residue from battery recycling waste. In this study, carrots had high bioaccumulation, measured as the percent of Pb concentration measured in the plant compared with the Pb concentration in the soil, with little translocation of the Pb to the shoots. Conversely, beets, cabbages, sweet peppers, and collard greens had low bioaccumulation but moderate to high translocation. Okra, tomatoes, and eggplants had moderate bioaccumulation and moderate to high translocation. Sesli et al. (2008) also noted uptake of Pb within wild mushrooms. Vandenhove et al. (2009) reviewed bioaccumulation data for plant groupings and found that grasses had the highest uptake, followed by leafy vegetables and root crops grown in sandy soils; see Table 3-6. These references also suggested high transfer from roots to shoots among root crops, with shoots having roughly four times higher Pb bioaccumulation than roots.

Sources of atmospheric Pb can lead to vegetable contamination. For example, Uzu et al. (2010) found that Pb deposition from smelter emissions caused a linear increase in Pb concentrations of 7.0 mg/kg per day (R^2=0.96) in lettuce plants cultivated in the courtyard of a smelter. They reported that lettuce grown 250-400 meters from the smelter had concentrations that were 10-20 times lower, which is consistent with findings described in Section 2.3 that deposition of Pb containing material drops off with distance from a source. Pb contamination of crops may also occur through piston-engine aircraft Pb emissions during aerial application of fertilizers and pesticides. In 2010, the U.S.

Federal Aviation Administration (FAA) recorded 396,000 hours of flight time for aerial application. This term encompasses crop and timber production including seeding cropland and fertilizer and pesticide application. It is estimated that 86% of these flight-hours involved piston engine aircraft utilizing leaded fuel (FAA, 2010).

Some land use and soil characteristics have been shown to increase bioaccessibility of Pb in soil, which could then lead to plant contamination. Fernandez et al. (2010; 2008; 2007) measured Pb from atmospheric deposition in two adjacent plots of land having the same soil composition but different uses: one was pasture land and one was agricultural. In the arable land, size distributions of soil particle-bound Pb, were uniformly distributed. In pasture land, size distributions of soil particle-bound Pb were bimodal with peaks around 2-20 μm and 50-100 μm (Fernandez et al., 2010). For the agricultural plot, Pb concentration was constant around 70 mg/kg in samples taken over the first 30 cm of soil, at which time it dropped below 10 mg/kg at soil depths between 35 and 100 cm. In contrast, Pb concentration in pasture land peaked at a depth of 10 cm at a concentration of roughly 70 mg/kg and then dropped off gradually to approach zero concentration at a depth of approximately 50 cm. The sharp change in concentration for the arable land was attributed to a combination of plowing the soil and use of fertilizers to increase the acidity of the soil and solubility of Pb into the soil (Fernandez et al., 2007). They found that the surface layer was acidic (pH: 3.37-4.09), as was the subsurface layer (pH: 3.65-4.38). Jin et al. (2005) examined how soil characteristics affect Pb contamination of crops by testing soil Pb, bioaccessibility of soil Pb (determined by $CaCl_2$ extraction), and Pb in tea samples from tea gardens. They observed that the Pb concentration in tea leaves was proportional to the bioaccessible Pb in soil.

There is some evidence that Pb contamination of crops can originate with treatment of crops. For example, compost produced from wastewater sludge has the potential to add Pb to crops. Cai et al. (2007) demonstrated that production of compost from sludge enriched the Pb content by 15-43% compared with the Pb content in sludge prior to composting. Chen et al. (2008b) observed that the median concentration of Pb in California crop soil samples was 16.2 mg/kg (range: 6.0-62.2 mg/kg). Chen et al. (2008a) further observed that in three of the seven California agricultural regions sampled, concentrations of Pb increased following addition of fertilizer, but the increase was less than that for phosphorous (P) and Zn indicators of fertilizer. In four regions, there was no increase of Pb at all. Furthermore, Tu et al. (2000) observed a decrease in Pb fraction with increasing P application. Nziguheba and Smolders (2008) also surveyed phosphate-based fertilizers sold in European markets to determine the contribution of these fertilizers to heavy metal concentrations in agricultural products. They reported a median fertilizer Pb concentration of 2.1 mg/kg based on total weight of the fertilizer, with a 95th percentile concentration of 7.5 mg/kg. Across Europe, Nziguheba and Smolders (2008)

estimated that the amount of Pb applied via fertilizers to be only 2.6% of that resulting from atmospheric deposition.

Although Pb in on-road vehicle gasoline has been phased out in the U.S., if imported crops are produced in countries that still use Pb antiknock agents in on-road gasoline, they have the potential to introduce dietary Pb to U.S. consumers. For example, high concentrations of Pb have been found in chocolate from beans grown in Nigeria, during the time when leaded gasoline was still legally sold. Rankin et al. (2005) observed that the ratios of ^{207}Pb to ^{206}Pb and ^{208}Pb to ^{207}Pb were similar to those of Pb in gasoline. Although this study showed that Pb concentration in the shelled cocoa beans was low (~1 ng/g), manufactured cocoa powder and baking chocolate had Pb concentrations similar to those of the cocoa bean shells, on the order of 200 ng/g, and Pb concentration in chocolate products was roughly 50 ng/g (Rankin et al., 2005). It is possible that the increases were attributed to contamination of the cocoa by the shells during storage or manufacture, but the authors note that more research is needed to verify the source of contamination.

Findings from Pb uptake studies have implications for urban gardening if urban soils may be contaminated with Pb. For instance, Clark et al. (2006) tested the soil in 103 urban gardens in two Boston neighborhoods. Using isotopic analysis, they found that Pb-based paint contributed 40-80% of Pb in the urban garden soil samples, with the rest coming from historical Pb emissions. Furthermore, Clark et al. (2006) estimated that Pb consumption from urban gardens can be equivalent to 10-25% of the exposure to Pb from drinking water for children living in the Boston neighborhoods studied. Because soil Pb levels in urban areas will depend on surrounding sources (Pruvot et al., 2006), Pb exposures in urban garden vegetables will vary.

Table 3-6 Pb bioaccumulation data for various plants. Bioaccumulation is expressed as percent of Pb concentration in the plant to the Pb concentration in the soil.

Plant Group	Plant Compartment	Soil	n	GM	GSD	AM	SD	Min	Max
All			210	2.0%	14	63%	290%	0.015%	2,500%
Cereals	Grain	All	9	1.0%	3.6	1.8%	1.6%	0.19%	4.8%
	Straw	All	4	2.3%	3.5	3.8%	4.0%	0.51%	9.6%
Maize	Grain	All	9	0.12%	2.3	0.17%	0.14%	0.052%	0.38%
	Straw	All	3	0.28%	6.6	0.85%	1.3%	0.060%	2.3%
Rice	Grain	All	2			2.2%	1.4%	1.2%	3.2%
Leafy Vegetables		All	31	8.0%	13	210%	610%	0.32%	2,500%
		Sand	4	7.3%	1.5	7.8%	3.3%	4.9%	11%
		Loam	3	82%	1.0	82%	3.5%	79%	86%
		Clay	7	2.8%	4.1	5.1%	4.8%	0.41%	12%
Non-Leafy Vegetables	Fruits	All	5	1.5%	26	78%	170%	0.15%	390%
	Shoots	All	2			0.88%	0.42%	0.58%	1.17%
Legumes	Pods	All	17	0.53%	12	34%	120%	0.046%	490%
		Sand	3	0.27%	3.2	0.42%	0.34%	0.065%	0.89%
		Loam	5	0.14%	4.4	0.42%	0.34%	0.065%	0.89%
		Clay	4	0.080%	1.0	0.33%	0.47%	0.046%	1.0%
	Shoots	All	1			0.080%			
Root Crops	Roots	All	27	1.5%	16	41%	98%	0.024%	330%
		Sand	5	6.4%	1.6	7.0%	3.4%	4.2%	12%
		Loam	5	2.3%	4.7	0.50%	0.68%	0.024%	1.7%
	Shoots	All	12	6.3%	15	250%	570%	0.30%	16%
Tubers	Tubers	All	30	0.15%	7.4	9.1%	48%	0.015%	260%
		Sand	5	0.64%	3.5	1.2%	1.6%	0.16%	3.9%
		Loam	17	0.052%	2.4	0.073%	0.062%	0.015%	0.23%
Fruits	Fruits	All	5	0.77%	2.6	1.0%	0.60%	0.15%	1.7%
	Leaves	All	1			25%			
Grasses		All	17	31%	1.8	36%	22%	11%	100%
Natural Pastures		All	34	92%	4.8	23%	29%	0.22%	100%
Leguminous Fodder		All	1			1.6%			
All Cereals		All	20	0.43%	4.7	1.1%	1.4%	0.052%	4.8%
		Sand	5	0.61%	5.3	1.3%	1.3%	0.052%	3.2%
		Loam	8	0.17%	3.9	0.53%	1.1%	0.059%	3.2%
		Clay	6	0.90%	4.0	1.8%	1.8%	0.22%	4.8%
Pastures/Grasses		All	51	14%	4.2	27%	27%	0.22%	100%
Fodder		All	24	2.5%	12	130%	420%	0.060%	1,600%
		Sand	4	4.5%	2.3	5.6%	4.0%	1.6%	11%
		Clay	4	0.82%	5.7	2.7%	4.6%	0.16%	9.6%

Source: Reprinted with permission of Elsevier Publishers, Vandenhove et al. (2009).

Game

Atmospheric sources of Pb have also been shown to contaminate game meat, thus potentially posing a risk of Pb exposure. In Pb mining or smelting areas, several studies have documented Pb concentrations in game [e.g., (Nwude et al., 2010; Reglero et al., 2009b)].

Potential Pb exposure through consumption of animals exposed to or killed with Pb shot has also been well documented (Hunt et al., 2009; Tsuji et al., 2009; Tsuji et al., 2008; Hunt et al., 2006). For example, Martínez-Haro et al. (2010) observed Pb in the feces of mallards that ingested gunshot of 34-13,930 mg/kg with a median of 1,104 mg/kg, while mallards that did not ingest gunshot had feces Pb levels <12.5 mg/kg. Mateo et al. (2011) studied Pb bioaccessibility as a function of cooking method for breast meat from partridges killed with gunshot. They observed that preparation in cold or hot vinegar increased bioaccessibility compared with total Pb in the samples.

Fish

Pb content in fish could also lead to human exposure to Pb (U.S. EPA, 2006b, 1986a). Ghosh et al. (2007) demonstrated in laboratory experiments that exposure to Pb in water can lead to linearly increasing Pb levels in the kidneys, liver, gills, skeleton, and muscle of fish. Several studies have documented the potential for human Pb exposure through fish and seafood. Welt et al. (2003) conducted a survey of individuals who fished in Bayou St. John, Louisiana in conjunction with sampling Pb content in sediment. They found that median sediment Pb concentrations ranged from 43 to 330 mg/kg in different locations, while maximum sediment Pb concentrations ranged from 580 to 6,500 mg/kg. In total, 65% of the surveyed individuals fished for food from the Bayou, with 86% consuming fish from the Bayou each week. In a study of the effect of coal mining on levels of metals in fish (measured as blood Pb) in northeastern Oklahoma, Schmitt et al. (2005) found that fish blood Pb levels varied with respect to species of fish, but blood Pb levels were higher in fish in areas close to mining activities. Similarly, Besser et al. (2008) observed higher levels of fish blood Pb, close to mining activities in southeastern Missouri. In a related study of fish species in the same region of Missouri, fish blood Pb levels were found to be statistically significantly higher in sites within 10 km downstream of active Pb-Zn mines ($p < 0.01$) compared with fish located further from the mines (Schmitt et al., 2007a), and elevated fish blood Pb levels were again noted near a Pb-Zn mine (Schmitt et al., 2009). It was noted that the Ozark streams where these studies were performed were often used for recreational fishing.

Breast Milk

Studies of breastfeeding women suggest that infants may be exposed to Pb in breast milk. Ettinger et al. (2004a) observed in a 1994-1995 study of Mexico City women that at 1 month postpartum, 88 women breastfeeding exclusively (with mean blood Pb level of 9.4 µg/dL) had breast milk Pb concentrations of 1.4 ± 1.1 µg/L, and 165 women breastfeeding partially (with mean blood Pb level of 9.5 µg/dL) had breast milk Pb concentrations of 1.5 ± 1.2 µg/L. During the same time period, Ettinger et al. (2006) studied breastfeeding women in Mexico City over a child's first year of life and sampled Pb concentration in breast milk at 1, 4, and 7 mo post-partum. They observed that mean breast milk concentrations dropped from 1.4 µg/L at 1 mo (mean maternal blood Pb = 9.3 µg/dL) to a mean of 1.2 µg/L at 4 mo (mean maternal blood Pb = 9.0 µg/dL) to 0.9 µg/L at 7 mo (mean maternal blood Pb = 8.1 µg/dL); this reduction was statistically significant ($p < 0.00001$). Among the 310 women included in the study, 181 had previous pregnancies. In one study of nursing mothers living in Port Pirie, Australia near a Pb smelter, 10 of the 11 mothers had breast milk concentrations <5 µg/L (Simon et al., 2007). The authors hypothesized that breast milk concentration was too low to be a major contributor to blood Pb level in these infants relative to other factors such as hand loading of Pb. However, one mother with a blood Pb level of 25 µg/dL had a breast milk Pb level of 28 µg/L (Simon et al., 2007).

In summary, several sources of dietary Pb can originate from atmospheric Pb emissions, including drinking water, vegetables, game, fish, and breast milk. Drinking water Pb levels are affected by source strength and proximity, runoff, and water treatment processes and chemicals. Among plants grown for agriculture, Pb content is highest in grasses, followed by leafy vegetables, then root vegetables. Pb in soil or dust can also collect on the surfaces of vegetables. Pb contamination of vegetables depends on a number of factors, including presence of nearby sources of atmospheric Pb, soil type and chemistry, land use, and land treatment. Other sources of Pb, such as international consumer products or historic emissions, also have the potential to introduce Pb into the U.S. diet. Pb contamination through the food chain potentially leads to elevated Pb levels in meat. Likewise, Pb contamination of surface waters can lead to elevated levels of Pb in fish used for consumption. Breastfeeding also presents a potential Pb exposure to newborn babies, and exposure drops off as the mothers nurse and as the babies age and add more food to their diet.

3.1.3.4 Occupational

Occupational environments have the potential to expose individuals to Pb. Some modern day occupational exposures are briefly discussed below in the context of understanding potential exposures that are not attributed to ambient air. For example, Miller et al. (2010) obtained personal and area samples of particle-borne Pb in a precious metals refinery; year of the study was not reported. It was not stated explicitly, but it is likely that Miller et al. (2010) measured the PM as TSP because the Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) for Pb is based on TSP rather than a smaller size cut, and the OSHA PEL was used for comparison. Concentrations measured by personal samples ranged from 2 to 6 $\mu g/m^3$, and concentrations from area samples ranged from 4 to 14 $\mu g/m^3$. The OSHA PEL is 5 $\mu g/m^3$. In steel production, sintering was found to be the largest source of airborne Pb exposure in a survey of operations (Sammut et al., 2010), with Pb enrichment in PM reported to be 20,000 mg/kg. Although total PM concentration was not reported by the authors, the PM was reported to have 75% of its particulate mass at below the 2.5 µm diameter size.

Operations involving Pb-containing materials in various industries are a source of occupational Pb exposure, in addition to a residential exposure. Rodrigues et al. (2010) reported exposures to airborne Pb among New England painters, who regularly use electric grinders to prepare surfaces for painting. Two-week averaged airborne Pb concentrations, sampled with an Institute of Medicine inhalable PM sampler designed to capture PM smaller than 100 µm, were reported to be 59 $\mu g/m^3$, with a maximum daily value of 210 $\mu g/m^3$. The Pb concentrations reported here were corrected by the National Institute for Occupational Safety and Health (NIOSH) respirator protection factors, although the respirator protection factors were not reported by Rodrigues et al. (2010). Information on the air Pb-blood Pb relationship can be found in Section 3.5.1.

3.1.3.5 Exposure to Pb from Consumer Products

Pb is present in varying amounts in several consumer products including alternative medicines, candies, cosmetics, pottery, tobacco, toys, and vitamins (Table 3-7). Several of these categories suggest children may incur regular exposures. Pb concentrations were reported to range from non-detectable levels up to 77% by mass, for the case of one medicinal product. Exposure to these products, which originate in a range of different countries, can account for substantial influence on Pb body burden (Miodovnik and Landrigan, 2009; Levin et al., 2008).

Table 3-7 Pb content in various consumer products.

Product Category	Product	Location of Purchase*	Pb Content (units)	Reference
Alternative and Traditional Medicines	Cissus quadrangularis, Caulophyllum thalictroides, Turnera diffusa, Centella asiatica, Hoodia gordonii, Sutherlandia frutescens, Curcuma longa, fucoxanthin, Euterpe oleracea (dietary supplements claimed to be from *Hoodia gordonii*)	U.S. (Mississippi)[a]	Not detected (N.D.)[b] to 4.21 mg/kg	Avula et al. (2010)
	Malva sylvestris	Turkey	1.1-2.0 mg/kg	Hiçsömnez et al. (2009)
	Yugmijihwang-tang, Bojungigki-tang, Sibjeondaebo-tang, Kuibi-tang, Ojeogsan	Korea	7.9×10^{-6} to 2.5×10^{-5} mg/kg body weight per day	Kim et al. (2009a)
	Lemongrass, licorice, holy basil, cloves, ginger	India	Average: Lemongrass & Holy Basil Leaves: 6.1 mg/kg; Licorice Stolons: 6.1 mg/kg, Clove Dried Flower Buds: 7.8 mg/kg, Ginger Rhizome: 5.8 mg/kg	Naithani and Kakkar (2006)
	B-Success 28, Operation Sweep, Aloe Vera Plus Bitter Aloes, Zarausmacine, Virgy-Virgy Computer Worm-Expeller, Dorasine Powder, Sexual Energy, U&DEE Infection Cleansing Powder, U&DEE Sweet Bitter, Natural Power Stone, Chama Black Stone, Portugal Antiseptic Soap, Edysol Antiseptic Soap, H-Nal, M-Reg, Veins Flocher, Diabor, C-Candi, C-Cysta, Firas, D-Diab, P-Pile, Infecta, Ribacin Forte, Aloe Vera Cure Formula	Nigeria	925-27,000 µg	Obi et al. (2006)
	Shell of Hen's Egg	India	14 mg/kg	Sharma et al. (2009)
	Berberis (B. aristata, B. chitria, B. asiatica, B. lyceum), Daruharidra	India	Berberis: Roots: 3.1-24.7 mg/kg Stems: 8.0-23.8 mg/kg Daruharidra: 16.9-49.8 mg/kg	Srivastava et al. (2006)
	Greta powder	U.S. (California)	770,000 ppm	CDC (2002)

Table 3-7 (Continued): Pb content in various consumer products.

Product Category	Product	Location of Purchase*	Pb Content (units)	Reference
Candy	Tamarind Candy	U.S. (Oklahoma)	Product: 0.15-3.61 mg/kg Stems: 0.36-2.5 mg/kg Wrappers: 459-27,125 mg/kg	Lynch et al. (2000)
	Tamarind Candy	U.S. (California)	Product: 0.2-0.3 mg/kg Stems: 400 mg/kg Wrappers: 16,000-21,000 mg/kg	CDC (2002)
Cosmetics	Lipsticks	U.S.	Average: 1.07 mg/kg	Hepp et al. (2009)
	Eye Shadows	Nigeria	N.D. to 55 mg/kg	Omolaoye et al. (2010a)
Pottery	Foods prepared in Pb-glazed pottery	Mexico	N.D. to 3,100 mg/kg	Villalobos et al. (2009)
Tobacco	Smokeless Tobacco	U.K.	0.15-1.56 mg/kg	McNeill et al. (2006)
	Cigarette Tobacco (^{210}Pb concentrations)	Pakistan	Activity conc.: 7-20 Bq/kg	Tahir and Alaamer (2008)
Toys	Red and yellow painted toy vehicles and tracks	Brazil	500-6,000 mg/kg	Godoi et al. (2009)
	535 PVC and non-PVC toys from day care centers	U.S. (Nevada)	PVC: avg. 325 mg/kg Non-PVC: avg. 89 mg/kg Yellow: 216 mg/kg Non-yellow: 94 mg/kg	Greenway and Gerstenberger (2010)
	Soft plastic toys	India	Average (by city): 21-280 mg/kg	Kumar and Pastore (2007)
	Toy necklace	U.S.	388,000 mg/kg	Meyer et al. (2008)
	Soft plastic toys	Nigeria	2.5-1,445 mg/kg	Omolaoye et al. (2010b)
Vitamins	Vitamins for young children, older children, and pregnant or lactating women	U.S.	Average: Young children: 2.9 µg/day Older children: 1.8 µg/day Pregnant and lactating women: 4.9 µg/day	Mindak et al. (2008)

[a]*Hoodia gordonii*, from Eastern Cape, South Africa *Euterpe oleracea* from Ninole Orchard, Ninole, Hawaii

*Note that the country of origin is not provided because it was not published in the references cited.

3.2 Kinetics

This section summarizes the empirical basis for understanding Pb toxicokinetics in humans. The large amount of empirical information on Pb biokinetics in humans and animal models has been integrated into mechanistic biokinetics models (U.S. EPA, 2006b). These models support predictions about the kinetics of Pb in blood and other selected tissues based on the empirically-based information about Pb biokinetics. In Section 3.3 (and Section 3.2.2.1), Pb biokinetics is described from the context of model predictions.

The discussion of Pb toxicokinetics emphasizes inorganic Pb since this comprises the dominant forms of Pb to which humans in the U.S. are currently exposed as a result of releases of Pb to the atmosphere and historic surface deposition of atmospheric Pb (see Section 2.2.2). The toxicokinetics of organic Pb is only briefly described and a more extensive discussion can be found in the 2006 Pb AQCD. Human exposures to organic Pb could occur in occupational settings (e.g., during manufacturing of tetraethyl Pb or aviation fuels); however, environmental exposures to organic Pb compounds rarely occur in the U.S. other than in the limited circumstances of those involved in fueling piston-driven aircraft that use leaded aviation gasoline.

3.2.1 Absorption

The major exposure routes of Pb in humans are inhalation and ingestion. Therefore, these exposure routes are important in the discussion of Pb absorption (see Section 3.2.1.1 and Section 3.2.1.2). The term “absorption” refers to the fraction of the amount of Pb ingested or inhaled that is absorbed from the respiratory or gastrointestinal tract. The term bioavailability, as it is used in this section, refers to the fraction of the amount of Pb ingested or inhaled that enters the systemic circulation. If properly measured (e.g., time-integrated blood Pb), under most conditions Pb bioavailability is equivalent (or nearly equivalent) to Pb absorption. The time-integrated blood Pb (i.e., the integral of blood Pb over time) provides a useful measure of bioavailability because it reflects both recent Pb absorption as well as contributions from Pb sequestered in soft tissue and bone. Bioaccessibility is a measure of the physiological solubility of Pb in the respiratory or gastrointestinal tract. Pb must become bioaccessible in order for absorption to occur. Processes that contribute to bioaccessibility include physical transformation of Pb particles and dissolution of Pb compounds into forms that can be absorbed (e.g., Pb^{2+}). Bioaccessibility is typically assessed by measuring the fraction of Pb in a sample that can

be extracted into a physiological or physiological-like solution (e.g., gastric juice or solution similar to gastric juice).

The 2006 Pb AQCD (U.S. EPA, 2006c) also presented dermal absorption of inorganic and organic Pb compounds, which is generally considered to be much less than by inhalation or ingestion. A study published subsequent to the 2006 Pb AQCD measured rates of absorption of Pb in skin patches harvested from nude mice (Pan et al., 2010). Following application of 12 mg Pb as Pb acetate or Pb nitrate, the absorption rate (measured over a 10-hour observation period) was approximately 0.02 µg Pb/cm^2 per hour. Absorbed Pb was detected in liver and kidney of nude mice following a 120-hr occluded dermal application of approximately 14 mg Pb as either Pb acetate or Pb nitrate. Uptake of Pb into the skin at the site of application was greater when Pb acetate was applied to the skin compared to Pb nitrate; however, liver and kidney Pb concentrations observed at the conclusion of the study (120 hours following the application of Pb) were not different for the two Pb compound. No additional information provides evidence of dermal absorption being a major exposure route of environmental Pb.

3.2.1.1 Inhalation

Systemic absorption of Pb deposited in the respiratory tract is influenced by particle size and solubility, as well as by the pattern of regional deposition within the respiratory tract. Fine particles (<1 µm) deposited in the bronchiolar and alveolar region can be absorbed after extracellular dissolution or can be ingested by phagocytic cells and transported from the respiratory tract (Bailey and Roy, 1994). Larger particles (>2.5 µm) that are primarily deposited in the ciliated airways (nasopharyngeal and tracheobronchial regions) can be transferred by mucociliary transport into the esophagus and swallowed, thus being absorbed via the gut.

Inhaled Pb lodging deep in the respiratory tract seems to be absorbed equally and totally, regardless of chemical form (Morrow et al., 1980; Chamberlain et al., 1978; Rabinowitz et al., 1977). Absorption half-times ($t_{1/2}$) have been estimated for radon decay progeny in adults who inhaled aerosols of Pb and bismuth isotopes generated from decay of ^{220}Rn or ^{222}Rn. The absorption half-time for Pb from the respiratory tract to blood was estimated to be approximately 10 hours in subjects who inhaled aerosols having an activity median particle diameter of approximately 160 nm (range 50-500 nm) (Marsh and Birchall, 1999), and approximately 68 min for aerosols having diameters of approximately 0.3–3 nm (Butterweck et al., 2002). Given the submicron particle size of the exposure, these rates are thought to represent, primarily, absorption from the bronchiolar and alveolar regions of the respiratory tract.

Several studies have quantified the bioaccessibility of Pb in atmospheric PM, based on various in vitro extraction methods. In a study of PM_{10} and $PM_{2.5}$ samples from downtown Vienna, Austria, Falta et al. (2008) used synthetic gastric juice to investigate the bioaccessibility of metals including Pb. The rationale was that inhaled particles in the 2.5-10 µm size range are mostly deposited in the tracheal and bronchial regions of the lung from where they are transported within hours by mucociliary clearance, i.e., they are mainly swallowed. In contrast, the <2.5 µm particles are deposited in the pulmonary alveoli where they can stay for months to years. The study aimed to determine the bioaccessibility of the 2.5-10 µm PM. It is important to note that they do not isolate the 2.5-10 µm size range; instead, they infer the characteristics from the difference between the $PM_{2.5}$ and PM_{10} fractions. The Pb concentrations associated with the two fractions were almost identical, as was the percentage extracted by synthetic gastric juice (86% and 83% Pb for $PM_{2.5}$ and PM_{10} fractions, respectively). The mean daily bioavailable mass was calculated to be 16 ng for the $PM_{2.5-10}$ size range. Since the quantitative clearance of these particles to the stomach was assumed, this value represents an upper estimate for the amount of bioavailable Pb. Niu et al. (2010) determined the bioaccessibility of Pb in fine (100-1,000 nm) and ultrafine-sized (<100 nm) urban airborne PM from two sites within the city of Ottawa, Canada. For all size fractions, the median Pb concentrations for particles smaller than 10 µm were 8,800 and 7,800 mg/kg for the two different locations. The bioaccessibility was based on ammonium acetate extractability and it was found that, within the fine and ultrafine-size ranges, 13-28% Pb was extracted. The Falta et al. (2008) and Niu et al. (2010) results illustrate that different extraction techniques result in different bioaccessible fractions. The main finding from Niu et al. (2010) was that the highest values (~28% and ~19% for the two different locations) were found for the <57 nm particles, with percent bioaccessibility decreasing with increasing particle size. This result indicated that Pb was potentially most bioaccessible in the ultrafine-size range.

A recent study by Barrett et al. (2010) investigated the solid phase speciation of Pb in urban road dust in Manchester, U.K., and considered the health implications of inhalation and ingestion of such material. Human exposure via inhalation is likely to involve only the finest grained fractions (up to 10 µm) and unfortunately this study characterized only the <38 µm fraction. Pb-goethite and $PbCrO_4$ comprised the largest fractions, 45% and 21% respectively, of Pb in the <38 µm fraction. These forms tend to be less bioaccessible if ingested compared with PbO or Pb acetate because they are less soluble.

The above considerations indicate that the relationship between air Pb exposure and blood Pb will depend on numerous exposure variables (e.g., particle size, solubility, exposure frequency and duration) and physiological variables (age, activity level, transport and absorption in the respiratory tract, blood Pb kinetics). For a detailed

discussion of factors affecting particle deposition and retention in the human respiratory tract the reader is referred to Chapter 4 of 2009 PM ISA (U.S. EPA, 2009a). Section 4.2.4 of that document specifically addresses biological factors affecting particle deposition such as activity level and age with an emphasis on children. Mechanistic models provide one means for integrating these variables into predictions of blood Pb – air Pb relationships; although, predictions are subject to simplifications and generalizations made in constructing the models. As an example, the ICRP (Pounds and Leggett, 1998; ICRP, 1994; Leggett, 1993) model (see Section 3.3 for a brief description) can be used to predict blood Pb – air Pb slopes for specific direct Pb inhalation exposure scenarios. For a long-term continuous (24 hours/day) exposure of a typical adult male engaged in light exercise (ventilation rate 20-22 m^3/day) to Pb-bearing particles having a 1 µm uniform particle size, the predicted blood Pb – air Pb slopes range from 0.7 µg/dL per µg/m^3 (for low solubility particles; e.g., Pb oxide) to 3 µg/dL per µg/m^3 (for highly soluble Pb; e.g., Pb salts). These slopes were calculated by running ICRP model simulations with varying air concentrations (0.1 - 6 µg/m^3) to achieve a range of blood Pb concentrations up to 10 µg/dL, starting with a baseline of 1.6 µg/dL, and estimating the linear slope of the relationship between blood Pb concentration and air Pb. Empirical estimates of blood Pb – air Pb slopes for various populations, derived from epidemiological studies, are summarized in Section 3.5.1.

Organic Pb

Alkyl Pb compounds can exist in ambient air as vapors. Inhaled tetraalkyl Pb vapor is nearly completely absorbed following deposition in the respiratory tract. As reported in the 2006 Pb AQCD (U.S. EPA, 2006c), a single exposure to vapors of radioactive (^{203}Pb) tetraethyl Pb resulted in 37% initially deposited in the respiratory tract, of which ~20% was exhaled in the subsequent 48 hours (Heard et al., 1979). In a similar experiment conducted with ^{203}Pb tetramethyl Pb, 51% of the inhaled ^{203}Pb dose was initially deposited in the respiratory tract, of which ~40% was exhaled in 48 hours (Heard et al., 1979).

Estimation of bioavailability of tetraethyl Pb following combustion is relevant to some aviation exposures (e.g., persons exposed to leaded gasoline used in piston-engine aircraft). Chamberlain et al. (1975) suggested that 35% of inhaled combustion products of tetraethyl ^{203}Pb fuel [likely to have been a mixture dominated by inorganic Pb halides, but may also have included alkly Pb species (U.S. EPA, 2006b)] are deposited and then retained in adult lungs with a half-life of 6 hours. Fifty percent of that ^{203}Pb was detectable in the blood within 50 hours of inhalation, and the rest was found to deposit in bone or tissue. Chamberlain et al. (1975) estimated that continuous inhalation of Pb in

engine exhaust from fuel containing tetraethyllead at a concentration of 1 μg/m^3 for a period of months could produce a 1 μg/dL increment in blood Pb.

3.2.1.2 Ingestion

The extent and rate of GI absorption of ingested inorganic Pb are influenced by physiological states of the exposed individual (e.g., age, fasting, nutritional calcium (Ca^{2+}) and iron (Fe) status, pregnancy) and physicochemical characteristics of the Pb-bearing material ingested (e.g., particle size, mineralogy, solubility). Pb absorption in humans may be a capacity-limited process, in which case the percentage of ingested Pb that is absorbed may decrease with increasing rate of Pb intake. Numerous observations of nonlinear relationships between blood Pb concentration and Pb intake in humans provide support for the likely existence of a saturable absorption mechanism or some other capacity-limited process in the distribution of Pb in humans (Sherlock and Quinn, 1986; Sherlock et al., 1984; Pocock et al., 1983; Sherlock et al., 1982). While evidence for capacity-limited processes at the level of the intestinal epithelium is compelling, the dose at which absorption becomes appreciably limited in humans is not known.

In adults, estimates of absorption of ingested water-soluble Pb compounds (e.g., Pb chloride, Pb nitrate, Pb acetate) range from 3 to 10% in fed subjects (Maddaloni et al., 1998; Watson et al., 1986; James et al., 1985; Heard and Chamberlain, 1982; Rabinowitz et al., 1980). The absence of food in the GI tract increases absorption of water-soluble Pb in adults. Reported estimates of soluble Pb absorption range from 26 to 70% in fasted adults (Maddaloni et al., 1998; James et al., 1985; Blake et al., 1983; Heard and Chamberlain, 1982; Rabinowitz et al., 1980). Reported fed:fasted ratios for soluble Pb absorption in adults range from 0.04 to 0.2 (James et al., 1985; Blake et al., 1983; Heard and Chamberlain, 1982; Rabinowitz et al., 1980).

Limited evidence demonstrates that GI absorption of water-soluble Pb is higher in children than in adults. Estimates derived from dietary balance studies conducted in infants and children (ages 2 weeks to 8 years) indicate that ~40-50% of ingested Pb is absorbed (Ziegler et al., 1978; Alexander et al., 1974). Experimental studies provide further evidence for greater absorption of Pb from the gut in young animals compared to adult animals (Aungst et al., 1981; Kostial et al., 1978; Pounds et al., 1978; Forbes and Reina, 1972). The mechanisms for an apparent age difference in GI absorption of Pb have not been completely elucidated and may include both physiological and dietary factors (Mushak, 1991). To further investigate the effects of the presence of food in the GI tract on Pb absorption, children (3-5 years old) who ate breakfast had lower blood Pb levels compared to children who did not eat breakfast (Liu et al., 2011a). This difference

persisted after controlling for nutritional variables (blood iron [Fe], calcium [Ca^{2+}], copper [Cu], magnesium [Mg], and zinc [Zn]). This observation may be explained by lower GI absorption of Pb ingested with or in close temporal proximity to meals. Direct evidence for meals lowering GI absorption of Pb has also been reported for adults (Maddaloni et al., 1998; James et al., 1985).

Nutritional interactions of Pb with dietary elements (e.g., Fe, Ca^{2+}, Zn) are complex. Pb competes with other elements for transport and binding sites that can result in adjustments of homeostatic regulators to absorb and retain needed elements. Additionally, low levels of macronutrients may alter Pb bioaccessibility in the GI tract. Genetic variation in absorption and metabolism may modify all of the above.

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

Several studies have suggested that dietary Ca^{2+} may have a protective role against Pb by decreasing absorption of Pb in the GI tract and by decreasing the mobilization of Pb from bone stores to blood. In experimental studies of adults, absorption of a single dose of Pb (100-300 µg Pb chloride) was lower when the Pb was ingested together with Ca^{2+} carbonate (0.2 g Ca^{2+} carbonate) than when the Pb was ingested without additional Ca^{2+} (Blake and Mann, 1983; Heard and Chamberlain, 1982). A similar effect of Ca^{2+} occurs in rats (Barton et al., 1978a). Similarly, an inverse relationship was observed between dietary Ca^{2+} intake and blood Pb concentration in children, suggesting that children who are Ca^{2+}-deficient may absorb more Pb than Ca^{2+}-replete children (Elias et al., 2007; Schell et al., 2004; Mahaffey et al., 1986; Ziegler et al., 1978). These

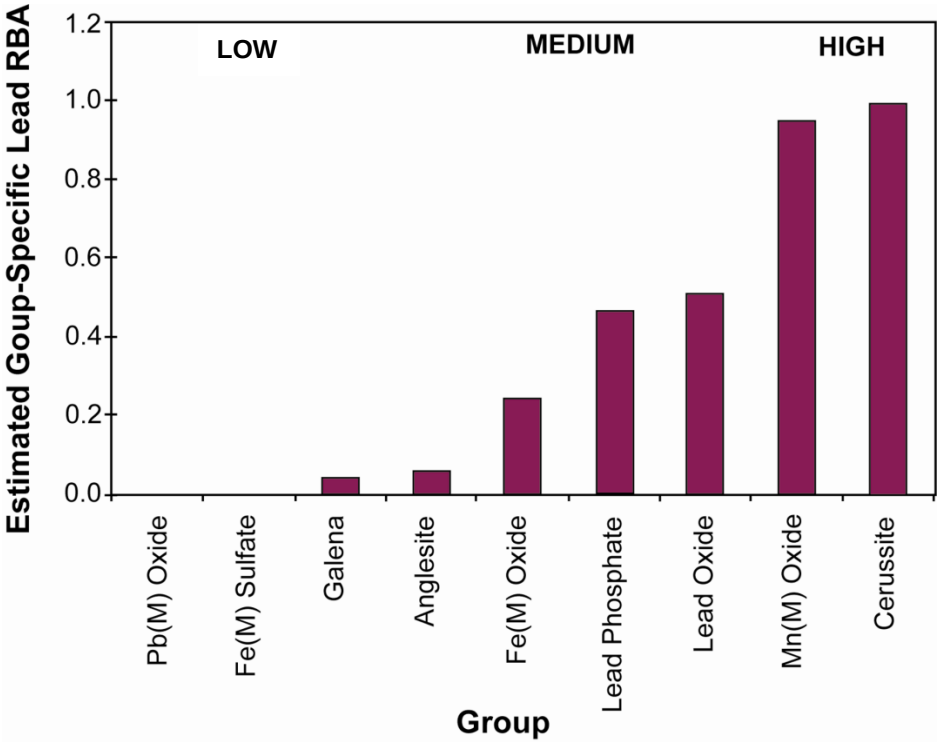
observations suggest that Ca^{2+} and Pb share and may compete for common binding and transport mechanisms in the small intestine which are regulated in response to dietary Ca^{2+} and Ca^{2+}-body stores (Fullmer and Rosen, 1990; Bronner et al., 1986). However, animal studies have also shown that multiple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

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

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

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

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



Note: based on results from juvenile swine assays.
Source: Casteel et al. (2006).

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

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

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

Equation 3-1

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

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

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

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

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

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

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

3.2.2 Distribution and Metabolism

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

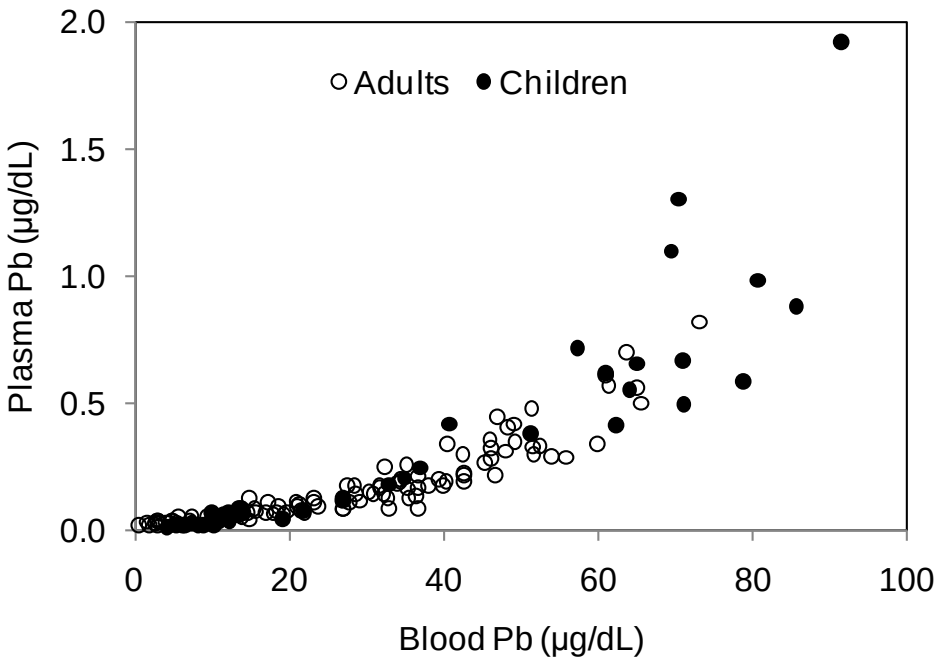
3.2.2.1 Blood

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

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

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

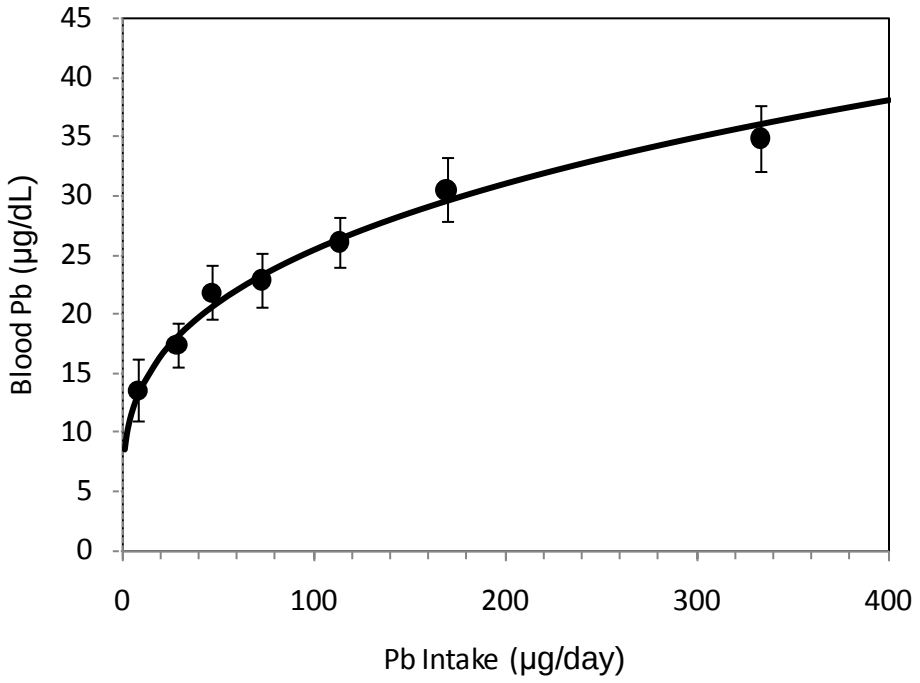
Typically, at blood Pb concentrations <100 µg/dL, only a small fraction (<1%) of blood Pb is found in plasma (Marcus, 1985; Manton and Cook, 1984; DeSilva, 1981). However, as previously noted, plasma Pb may be the more biologically labile and toxicologically effective fraction of the circulating Pb.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



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

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

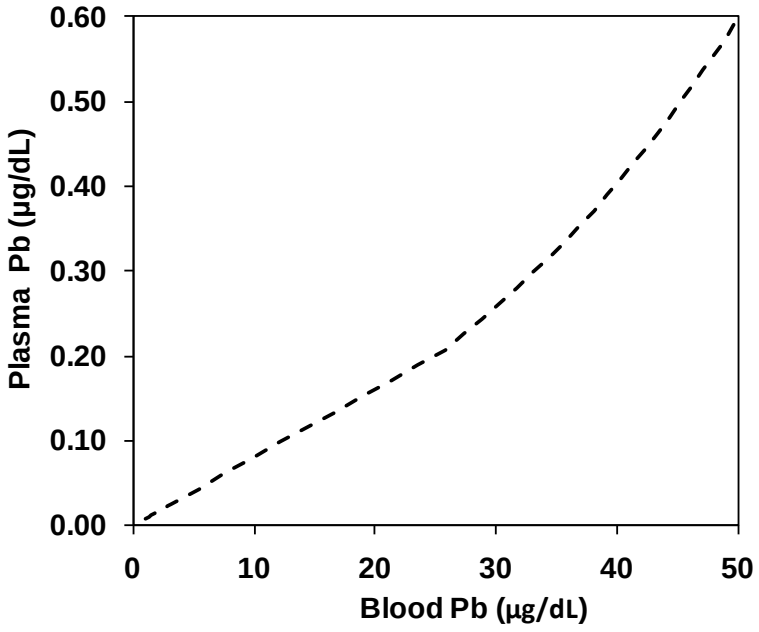
As shown in Figure 3-4, the limited binding capacity of Pb binding proteins in RBCs produces a curvilinear relationship between blood and plasma Pb concentration. The limited binding capacity of RBC binding proteins also confers, or at least contributes, to a curvilinear relationship between Pb intake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

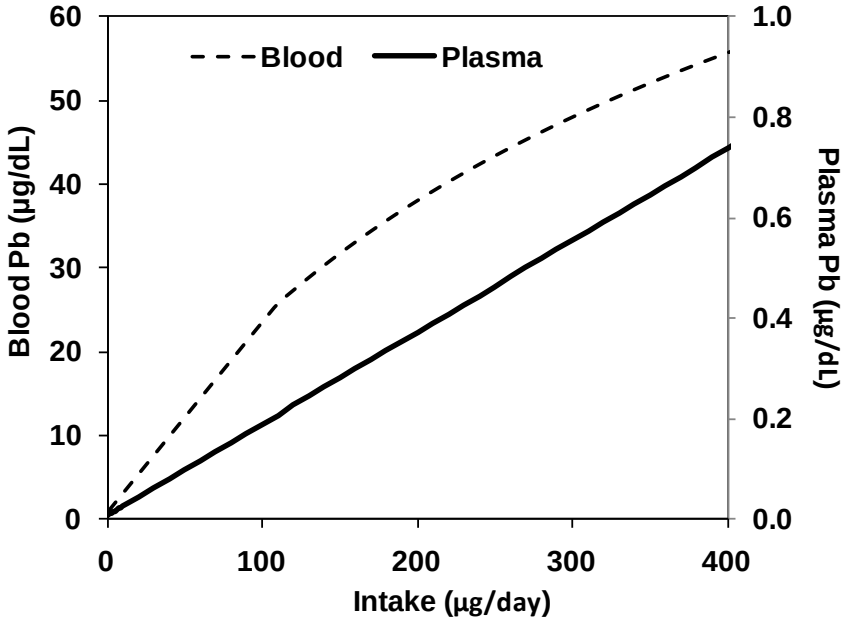


Data represent mean and standard errors for intake; the line is the regression model (blood Pb = 3.9 + 2.43 (Pb intake [µg/week]$^{1/3}$).
Source: Adapted with permission of Taylor & Francis Publishing, Sherlock and Quinn (1986).

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

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





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

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

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

3.2.2.2 Bone

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

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

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

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

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

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

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

3.2.2.3 Soft Tissues

Most of the Pb in soft tissue is in liver and kidney (Gerhardsson et al., 1995; Oldereid et al., 1993; Gerhardsson et al., 1986; Barry, 1981, 1975; Gross et al., 1975). Presumably, the Pb in these soft tissues (i.e., kidney, liver, and brain) exists predominantly bound to protein. High affinity cytosolic Pb-binding proteins have been identified in rat kidney and brain (DuVal and Fowler, 1989; Fowler, 1989). The Pb-binding proteins in rat are cleavage products of α2μ globulin, a member of the protein superfamily known as retinol-binding proteins that are generally observed only in male rats (Fowler and DuVal, 1991). Other high-affinity Pb-binding proteins (K_d ~14 nM) have been isolated in human kidney, two of which have been identified as a 5 kDa peptide, thymosin 4 and a 9 kDa peptide, acyl-CoA binding protein (Smith et al., 1998). Pb also binds to metallothionein, but does not appear to be a significant inducer of the protein in comparison with the inducers Cd and Zn (Waalkes and Klaassen, 1985; Eaton et al., 1980).

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

3.2.2.4 Fetus

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

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

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

3.2.2.5 Organic Pb

Information on the distribution of Pb in humans following exposures to organic Pb is extremely limited. However, as reported in the 2006 Pb AQCD (U.S. EPA, 2006c), the available evidence demonstrates near complete absorption following inhalation of tetraalkyl Pb vapor and subsequent transformation to trialkyl Pb metabolites. One hour following brief inhalation exposures to ^{203}Pb tetraethyl or tetramethyl Pb (1 mg/m^3), ~50% of the ^{203}Pb body burden was associated with liver and 5% with kidney; the remaining ^{203}Pb was widely distributed throughout the body (Heard et al., 1979). The kinetics of ^{203}Pb in blood showed an initial declining phase during the first 4 hours (tetramethyl Pb) or 10 hours (tetraethyl Pb) after the exposure, followed by a reappearance of radioactivity back into the blood after ~20 hours. The high level of radioactivity initially in the plasma indicates the presence of tetraalkyl/trialkyl Pb. The subsequent rise in blood radioactivity, however, probably represents water-soluble inorganic Pb and trialkyl and dialkyl Pb compounds that were formed from the metabolic conversion of the volatile parent compounds (Heard et al., 1979).

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

3.2.3 Elimination

The rapid-phase (30-40 days) of Pb excretion amounts to 50-60% of the absorbed fraction (Chamberlain et al., 1978; Rabinowitz et al., 1976; Kehoe, 1961a, b, c). Absorbed Pb is excreted primarily in urine and feces, with sweat, saliva, hair, nails, and breast milk being minor routes of excretion (Kehoe, 1987; Chamberlain et al., 1978; Rabinowitz et al., 1976; Griffin et al., 1975; Hursh et al., 1969; Hursh and Suomela, 1968).

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

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

Estimates of urinary filtration of Pb from plasma range from 13-22 L/day, with a mean of 18 L/day (Araki et al., 1986; Manton and Cook, 1984; Manton and Malloy, 1983; Chamberlain et al., 1978), which corresponds to half-time for transfer of Pb from plasma to urine of 0.1-0.16 days for a 70-kg adult who has a plasma volume of ~3 L. The rate of urinary excretion of Pb was less than the rate of glomerular filtration of ultrafilterable Pb, suggesting that urinary Pb is the result of incomplete renal tubular reabsorption of Pb in the glomerular filtrate (Araki et al., 1986); although, net tubular secretion of Pb has been demonstrated in animals (Victery et al., 1979; Vander et al., 1977). On the other hand, estimates of blood-to-urine clearance range from 0.03-0.3 L/day with a mean of 0.18 L/day (Diamond, 1992; Araki et al., 1990; Berger et al., 1990; Koster et al., 1989; Manton and Malloy, 1983; Ryu et al., 1983; Chamberlain et al., 1978; Rabinowitz et al., 1973), consistent with a plasma Pb to blood Pb concentration ratio of ~0.005–0.01 L/day (Klotzback et al., 2003). Based on the above differences, urinary excretion of Pb can be expected to reflect the concentration of Pb in plasma and variables that affect delivery of Pb from plasma to urine (e.g., glomerular filtration and other transfer processes in the kidney).

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

Organic Pb

Pb absorbed after inhalation of tetraethyl and tetramethyl Pb is excreted in exhaled air, urine, and feces (Heard et al., 1979). Fecal:urinary excretion ratios were 1.8 following exposure to tetraethyl Pb and 1.0 following exposure to tetramethyl Pb (Heard et al., 1979). Occupational monitoring studies of workers exposed to tetraethyl Pb showed that tetraethyl Pb is excreted in the urine as diethyl Pb, ethyl Pb, and inorganic Pb (Vural and Duydu, 1995; Zhang et al., 1994; Turlakiewicz and Chmielnicka, 1985).

3.3 Pb Biomarkers

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

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

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

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

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

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

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

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

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

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

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

3.3.1 Bone-Pb Measurements

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

Bone Pb measurements are typically expressed in units of μg Pb per g bone mineral. This convention may potentially introduce variability into the bone Pb measurements related to variation in bone density. Typically, potential associations between bone density and bone Pb concentration are not evaluated in epidemiologic studies (Theppeang et al., 2008a; Hu et al., 2007a). An important consequence of expressing bone Pb measures relative to bone mineral content is that lower bone mineral density is associated with greater measurement uncertainty in bone Pb. This uncertainty can have important implications for studies in older women for whom low bone mineral density is more common than in other populations including men and younger adults.

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

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

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

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

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

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

3.3.2 Blood-Pb Measurements

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

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

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

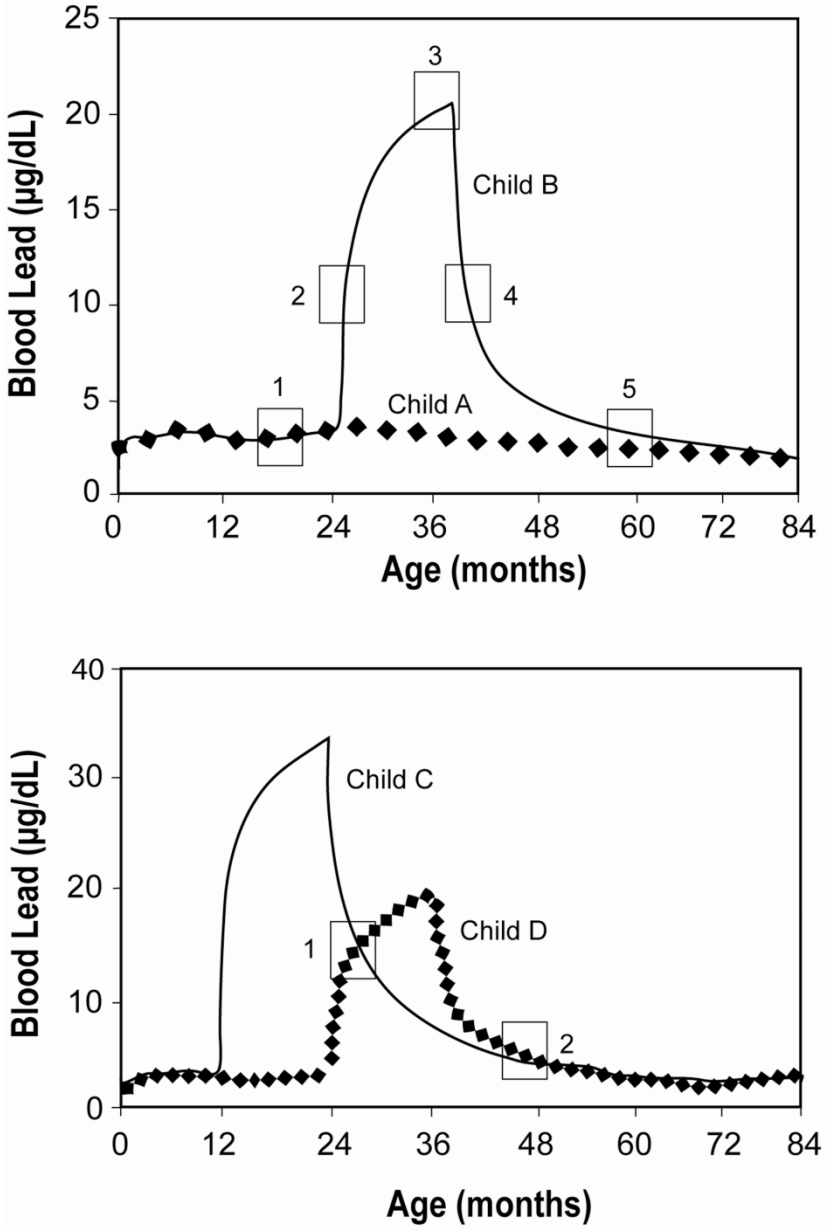
The 2006 Pb AQCD (U.S. EPA, 2006c) reported that many studies have shown that, with similar exposures to Pb, individuals with the ALAD-2 allele have higher blood Pb levels

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

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

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

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



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

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

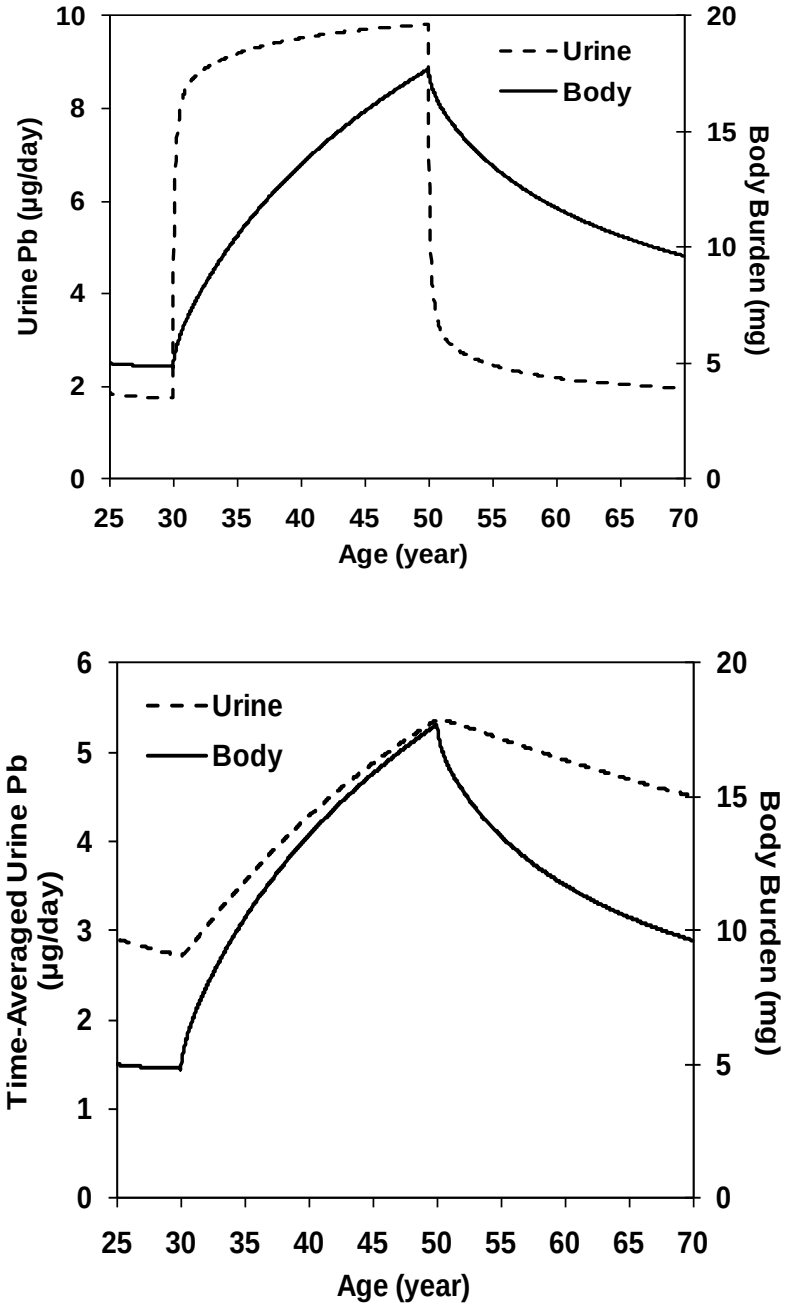
3.3.3 Urine-Pb Measurements

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

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

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

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



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

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

3.3.4 Pb in Other Biomarkers

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

3.3.4.1 Teeth

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

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

3.3.4.2 Hair

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

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

3.3.4.3 Saliva

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

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

3.3.4.4 Serum δ-ALA and ALAD

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

3.3.5 Relationship between Pb in Blood and Pb in Bone

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

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

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

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

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

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

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

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

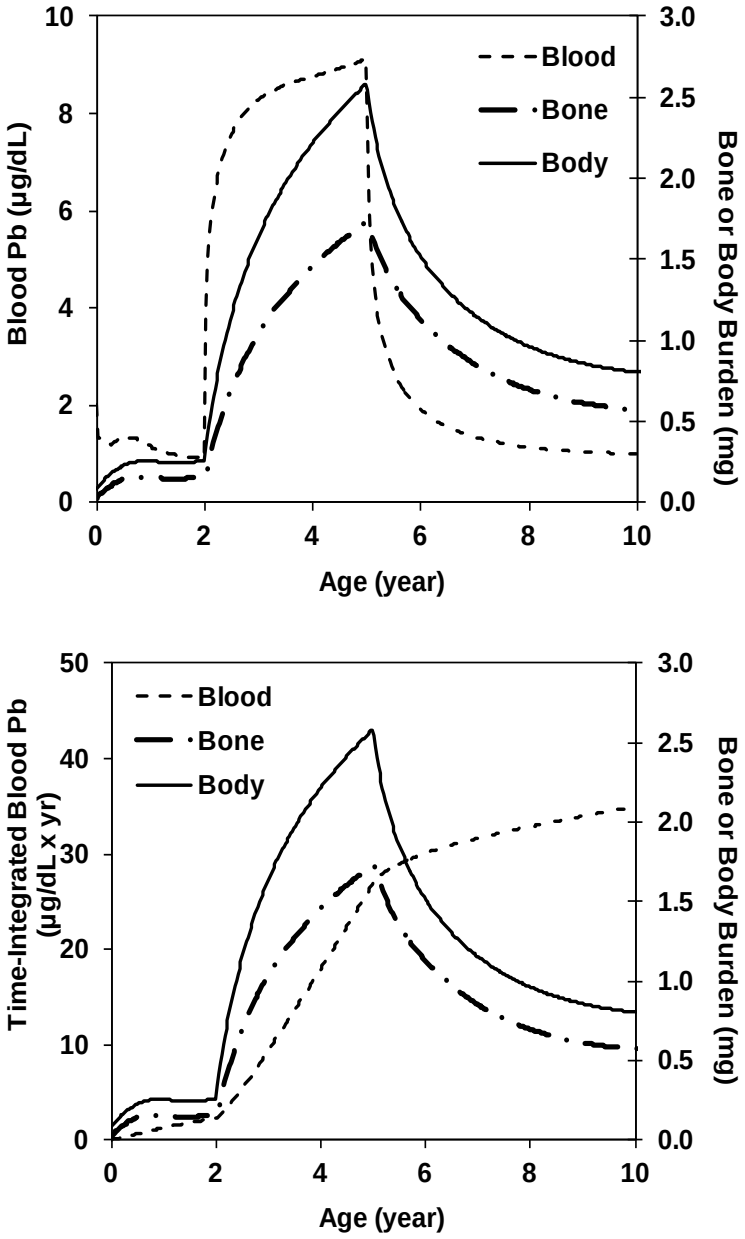
3.3.5.1 Children

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

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

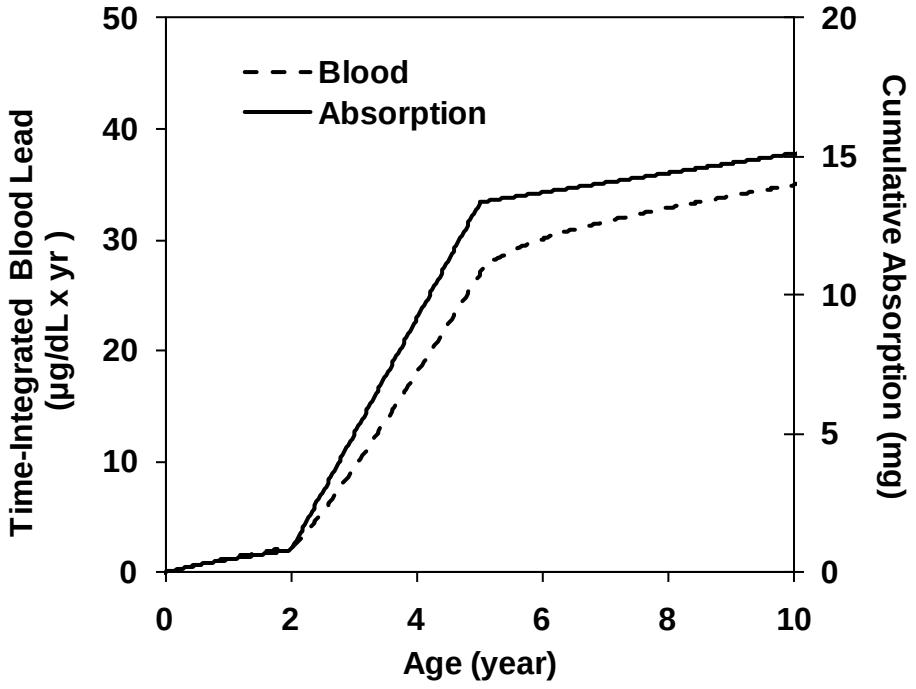
Time-integrated blood Pb metrics display rates of change in response to the exposure event that more closely approximate the slower kinetics of total skeletal bone Pb and body burden, than the kinetics of blood Pb concentration, with notable differences (Figure 3-9). The time-integrated blood Pb concentration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

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



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

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



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

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

3.3.5.2 Adults

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

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

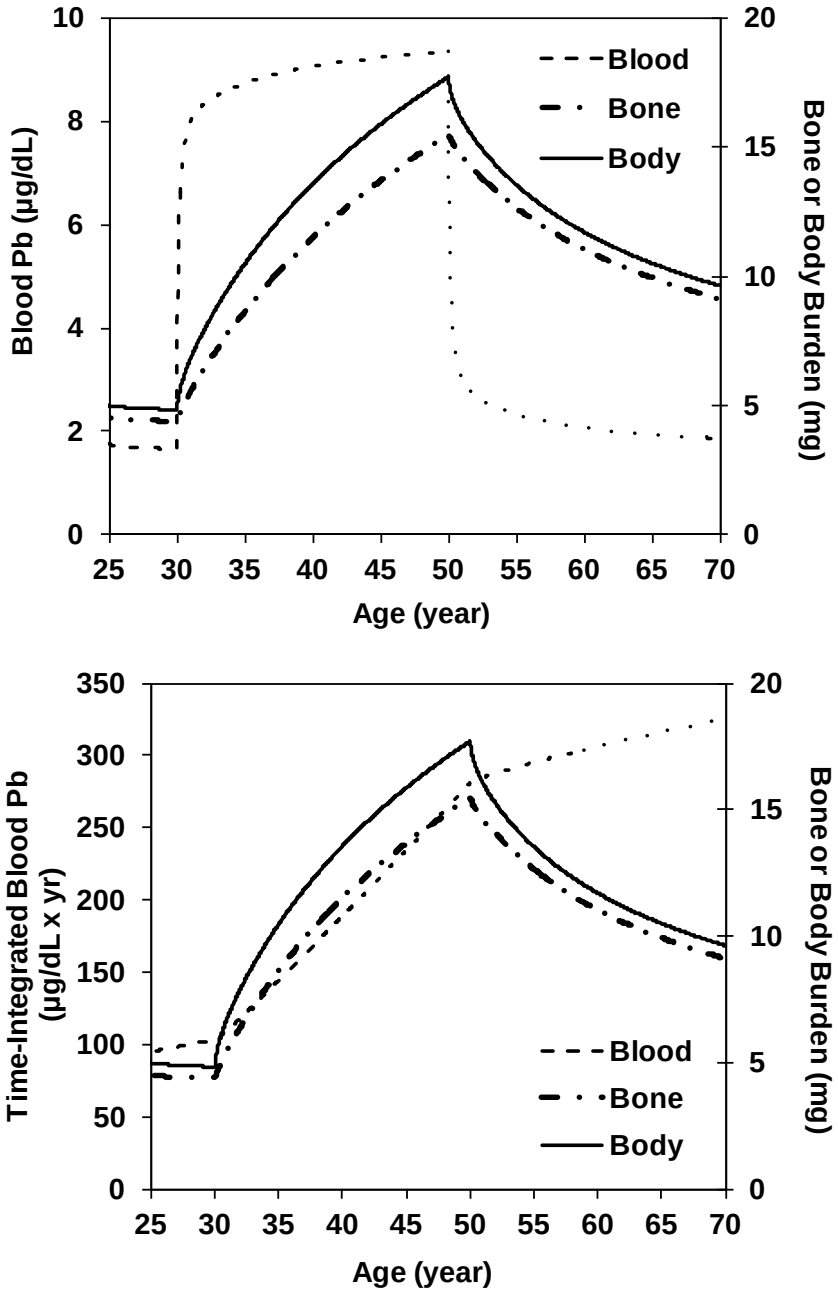
The simulation shown in Figure 3-11 represents an exposure that resulted in a quasi-steady state blood Pb concentration of approximately 10 µg/dL. Exposures that achieve higher blood Pb concentrations, more indicative of poisoning or historic occupational exposures will result in a more prolonged elevation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

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

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

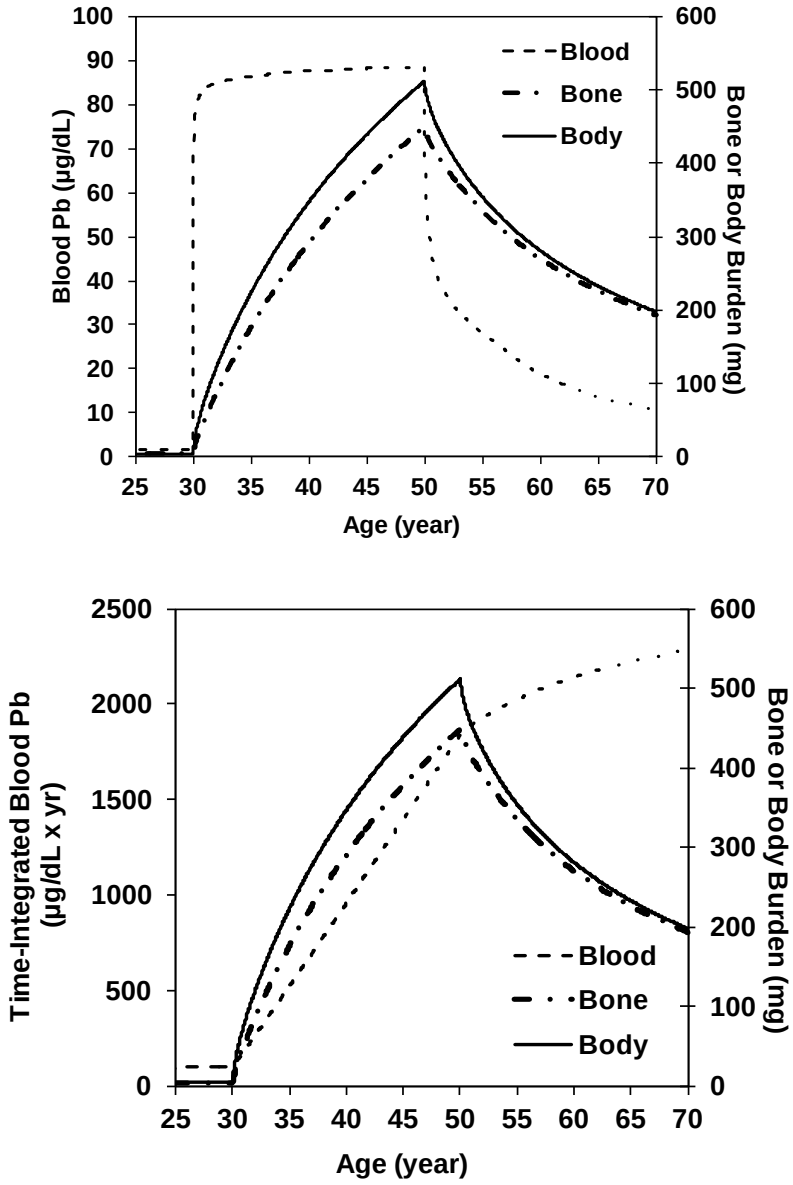
will diverge with increasing exposure duration and age. This divergence can impart different degrees of age-confounding when either blood Pb or bone Pb is used as an internal dose metric in dose-response models. In a review of epidemiologic studies that evaluated the associations between blood Pb, bone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

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



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

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



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

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

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

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

Mobilization of Pb from Bone in Adulthood

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

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

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

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

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

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

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

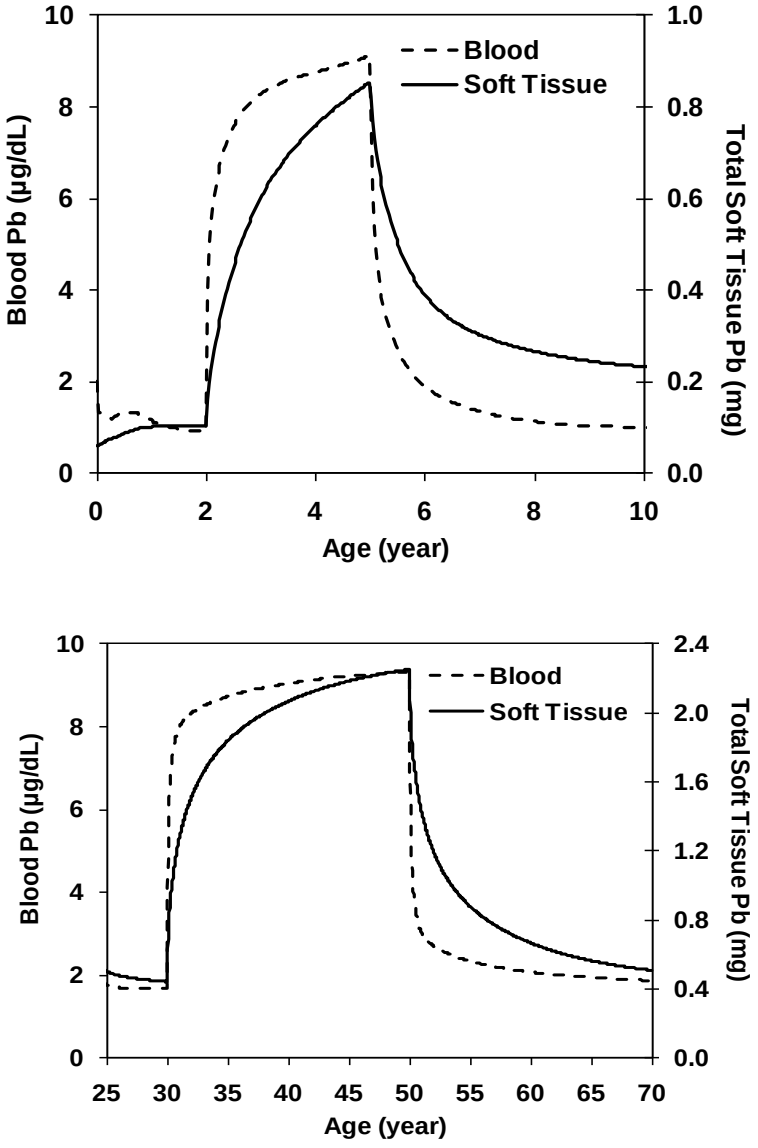
The Pb content in some bones (i.e., mid femur and pelvic bone) plateau at middle age and then decreases at older ages (Drasch et al., 1987). This decrease is most pronounced in females and may be due to osteoporosis and release of Pb from resorbed bone to blood (Gulson et al., 2002). Two studies indicate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

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

3.3.6 Relationship between Pb in Blood and Pb in Soft Tissues

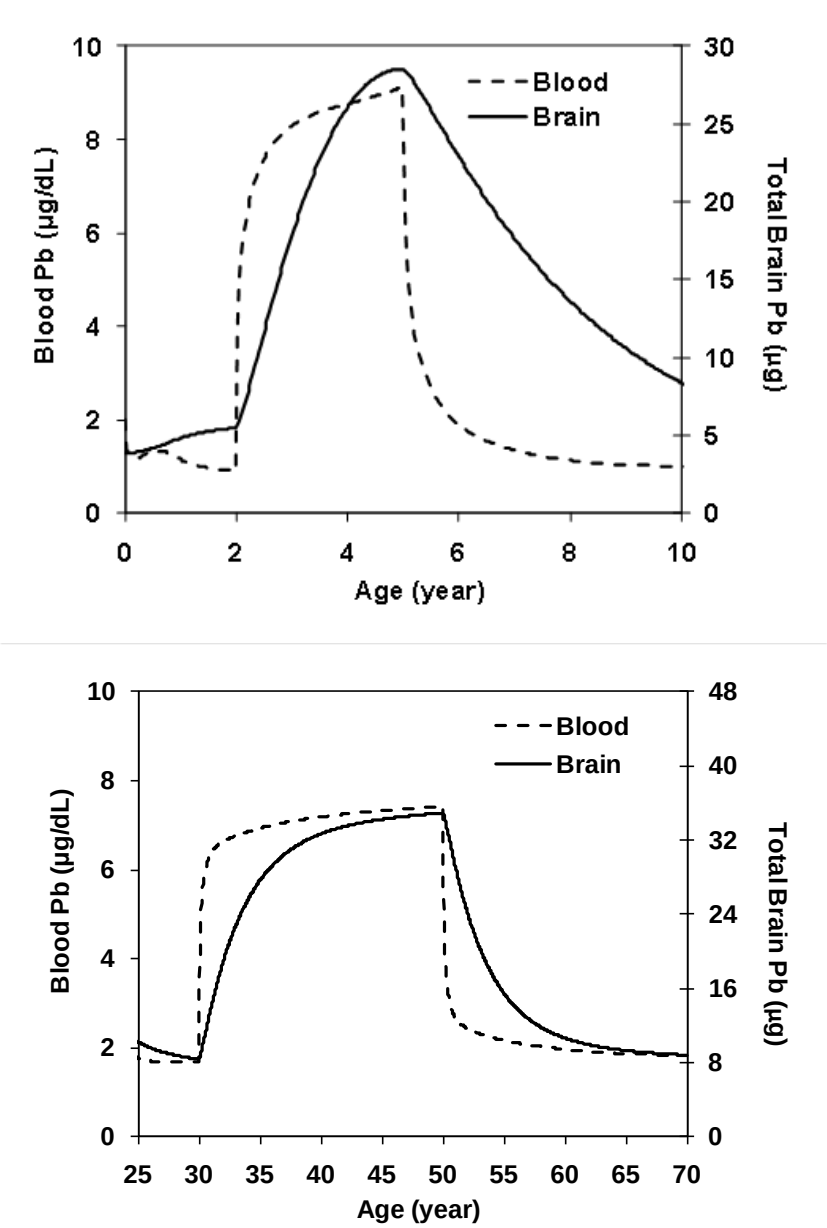
Figure 3-13 shows simulations of blood and soft tissues Pb (including brain) for the same exposure scenarios previously displayed. Pb uptake and elimination in soft tissues is much faster than bone. As a result, following cessation of a period of elevated exposure, Pb in soft tissues is more quickly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

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



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

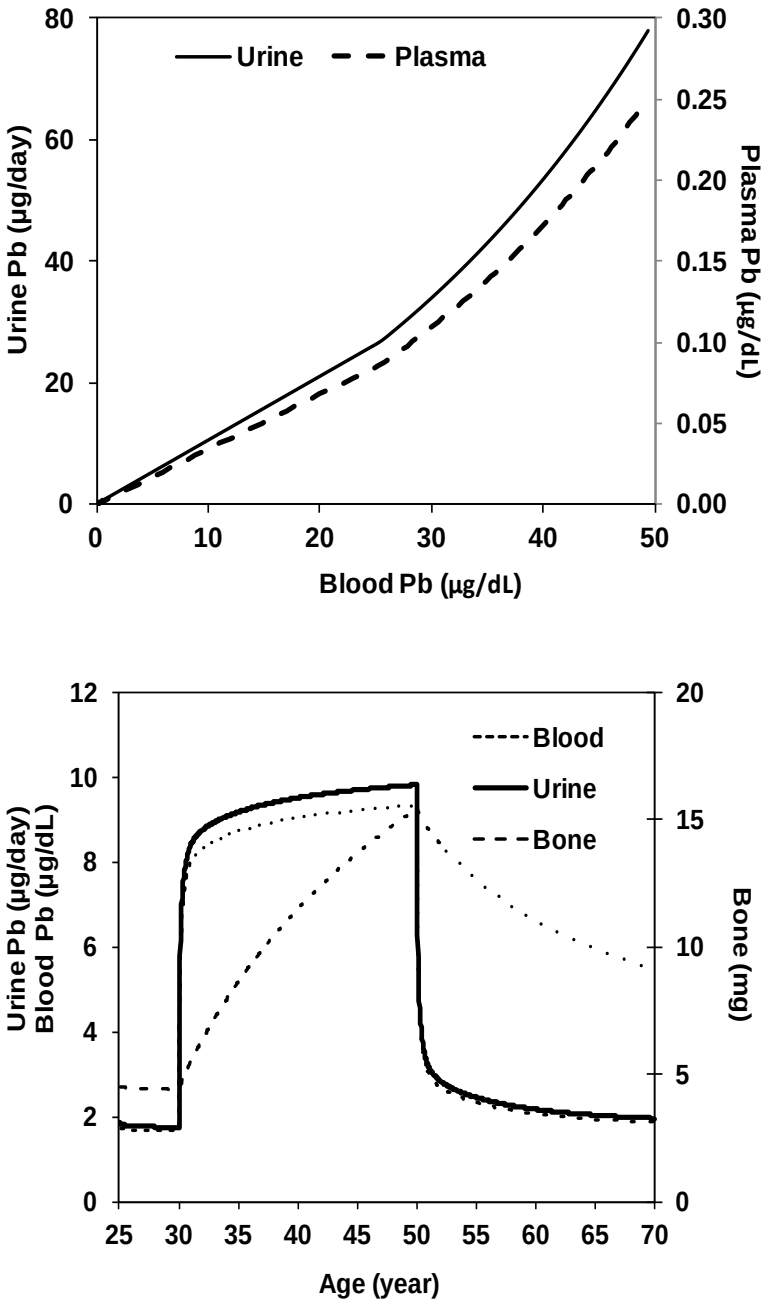
Figure 3-13 Simulation of blood and soft tissue (including brain) Pb in children and adults who experience a period of increased Pb intake.



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

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

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



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

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

3.4 Studies of Pb Biomarker Levels

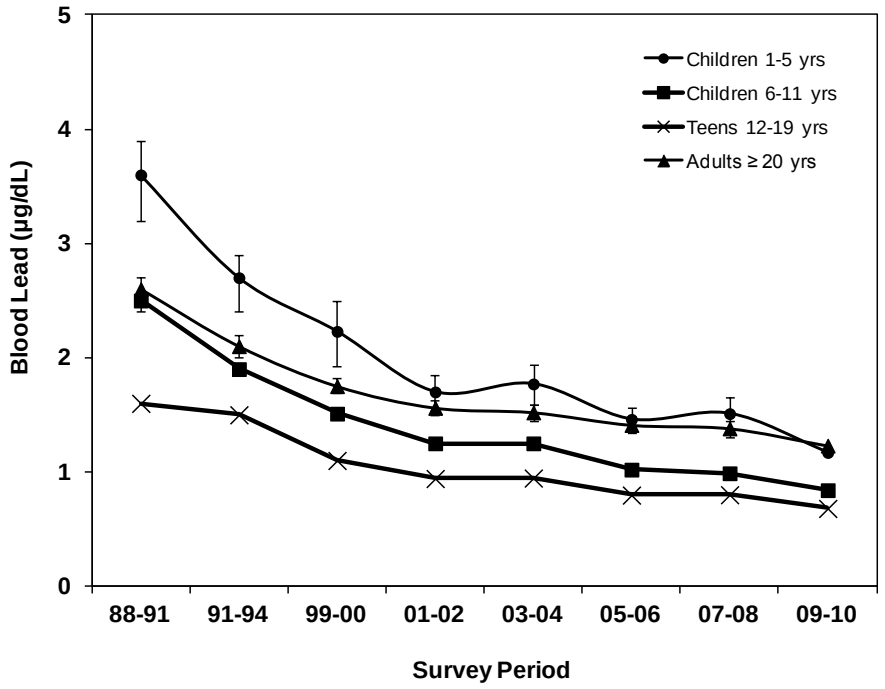
3.4.1 Pb in Blood

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

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

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

When race/ethnicity groups were compared for years 1999-2004, geometric means (GM) of blood Pb levels in children were highest in the ethnicity category non-Hispanic black (GM 2.8, 95% CI: 2.5, 3.0) compared to the categories Mexican-American (GM 1.9, 95% CI: 1.7, 2.0) and non-Hispanic white (GM 1.7, 95% CI: 1.6, 1.8) (Jones et al., 2009a). Figure 3-19 demonstrates the change in percent of children (aged 1-5 years) with various blood Pb levels by race/ethnicity between the survey during 1988-1991 and that during 1999-2004. When these data for children aged 1-5 years were aggregated for all survey years from 1988 to 2004, residence in older housing, poverty, age, and being non-Hispanic black were significant predictors of higher Pb levels (Jones et al., 2009a).



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

Figure 3-16 Temporal trend in blood Pb concentration.

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

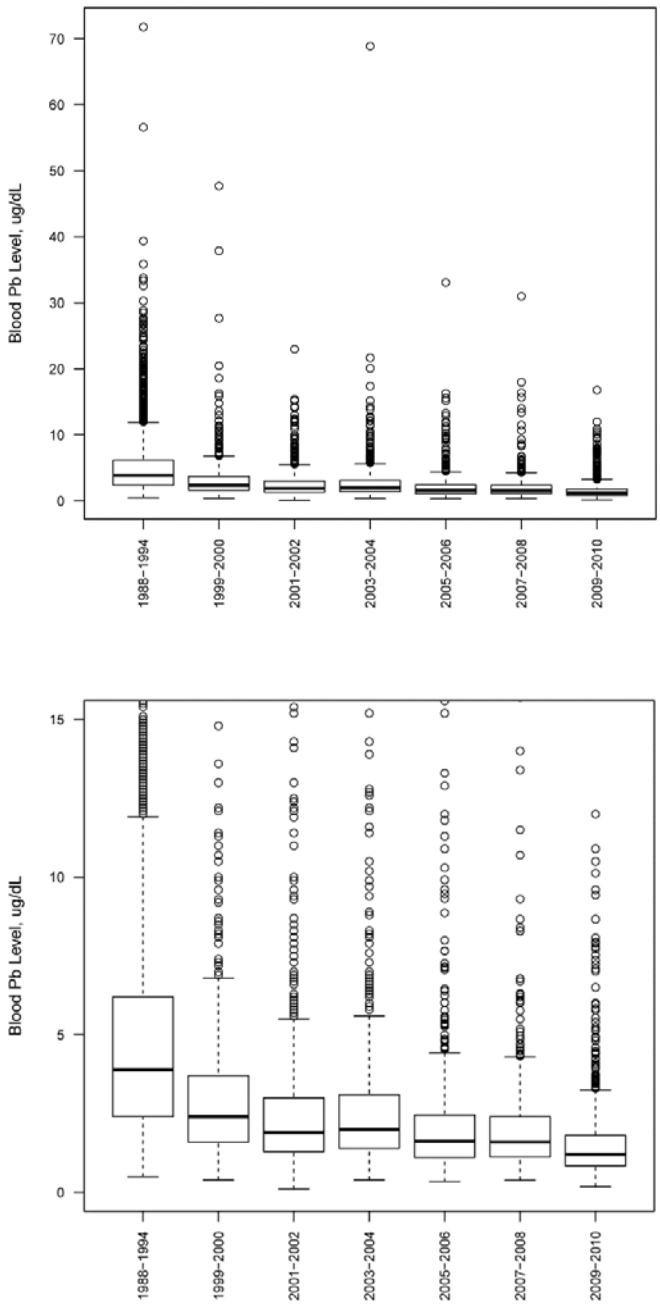
Survey Stratum	Period	Geometric Mean (µg/dL)	95% Confidence Interval	Number of Subjects
All	1999-2000	1.66	1.60, 1.72	7,970
	2001-2002	1.45	1.39, 1.51	8,945
	2003-2004	1.43	1.36, 1.50	8,373
	2005-2006	1.29	1.23, 1.36	8,407
	2007-2008	1.27	1.21, 1.34	8,266
	2009-2010	1.12	1.08, 1.16	8,793
1-5 yr	1999-2000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

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

Survey Stratum	Period	Geometric Mean (µg/dL)	95% Confidence Interval	Number of Subjects
Males	1999-2000	2.01	1.93, 2.09	3,913
	2001-2002	1.78	1.71, 1.86	4,339
	2003-2004	1.69	1.62, 1.75	4,132
	2005-2006	1.52	1.42, 1.62	4,092
	2007-2008	1.47	1.39, 1.56	4,147
	2009-2010	1.31	1.25, 1.36	4,366
Females	1999-2000	1.37	1.32, 1.43	4,057
	2001-2002	1.19	1.14, 1.25	4,606
	2003-2004	1.22	1.14, 1.31	4,241
	2005-2006	1.11	1.05, 1.17	4,315
	2007-2008	1.11	1.06, 1.16	4,119
	2009-2010	0.97	0.93, 1.01	4.427
Mexican – Americans	1999-2000	1.83	1.75, 1.91	2,742
	2001-2002	1.46	1.34, 1.60	2,268
	2003-2004	1.55	1.43, 1.69	2,085
	2005-2006	1.29	1.21, 1.38	2,236
	2007-2008	1.25	1.15, 1.36	1,712
	2009-2010	1.14	1.03, 1.28	1,966
Non-Hispanic blacks	1999-2000	1.87	1.75, 2.00	1,842
	2001-2002	1.65	1.52, 1.80	2,219
	2003-2004	1.69	1.52, 1.89	2,293
	2005-2006	1.39	1.26, 1.53	2,193
	2007-2008	1.39	1.30, 1.48	1,746
	2009-2010	1.24	1.18, 1.30	1,593
Non-Hispanic whites	1999-2000	1.62	1.55, 1.69	2,716
	2001-2002	1.43	1.37, 1.48	3,806
	2003-2004	1.37	1.32, 1.43	3,478
	2005-2006	1.28	1.19, 1.37	3,310
	2007-2008	1.24	1.16, 1.33	3,461
	2009-2010	1.10	1.04, 1.16	3,760

Age strata correspond to the NHANES study design.

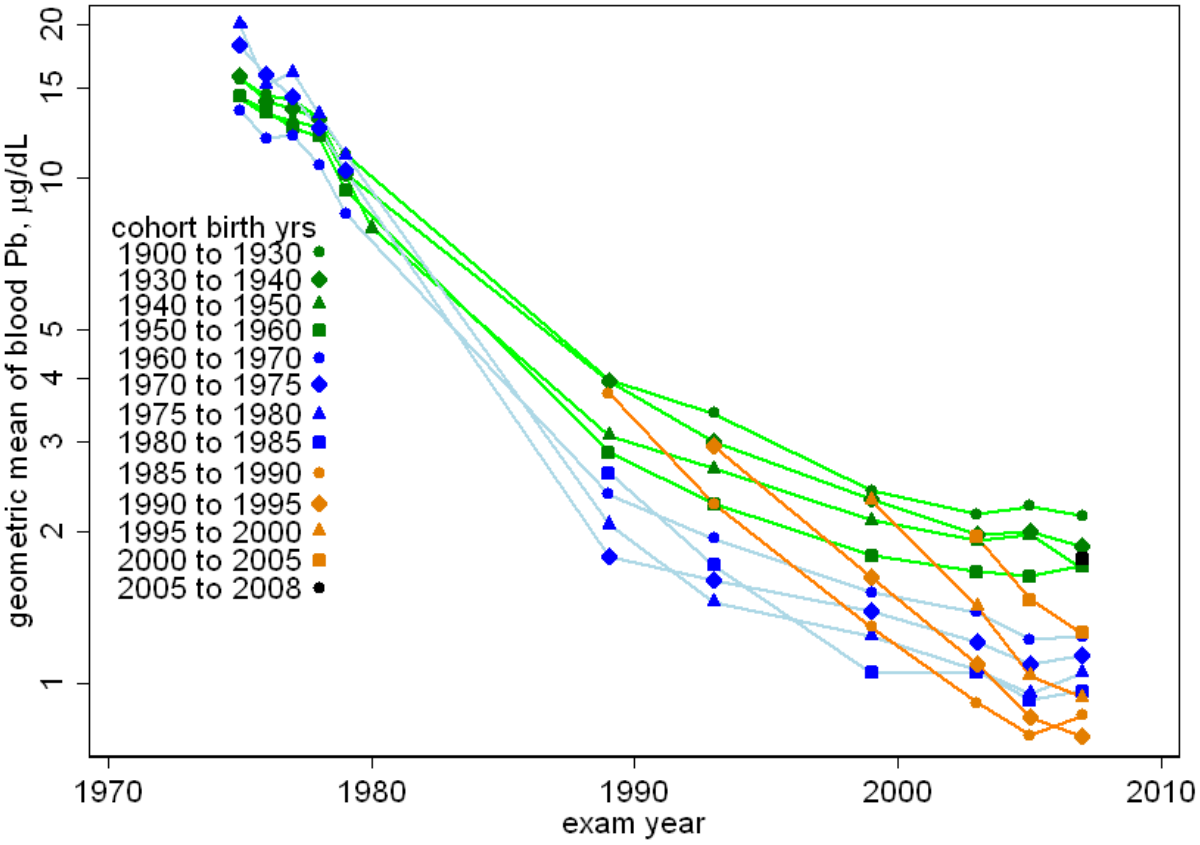
Source: Adapted from data from the NHANES (CDC, 2013).



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

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

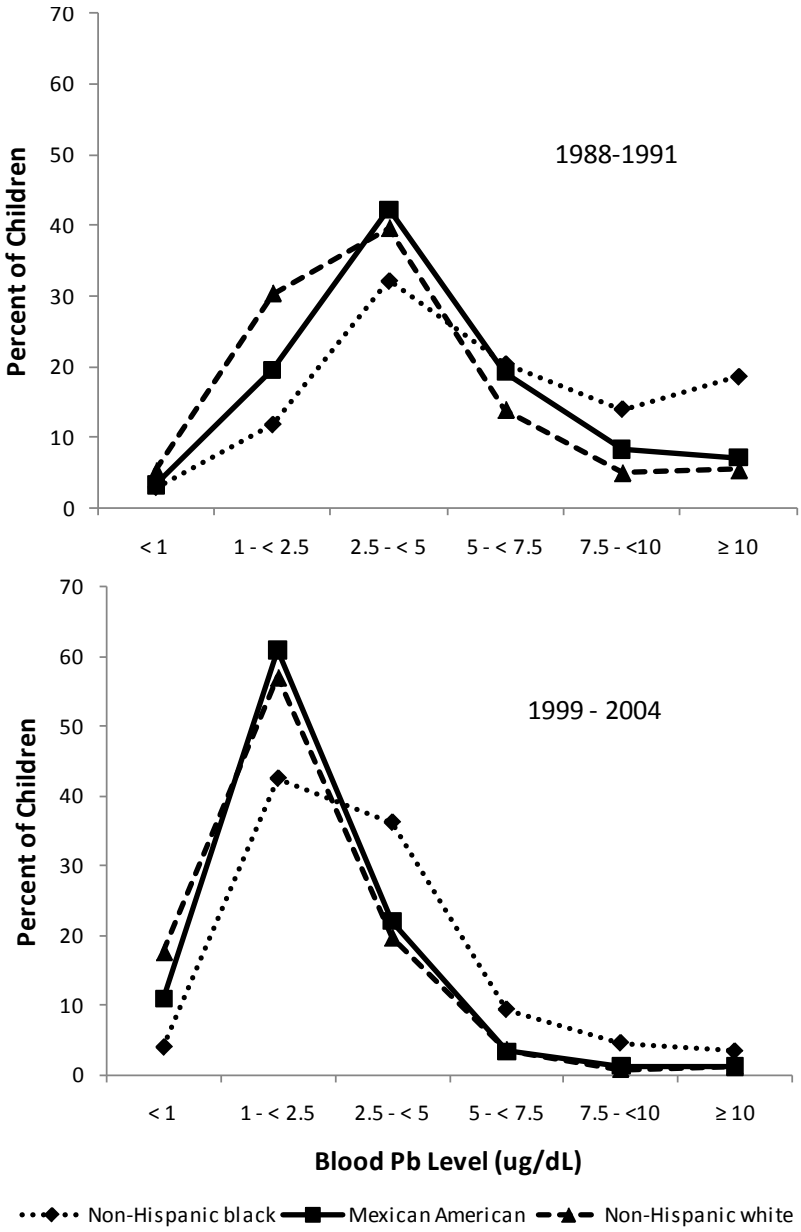
Figure 3-17 Box plots of blood Pb levels among U.S. children (1-5 years old) from the NHANES survey, 1988-2010.



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

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

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



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

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

In agreement with the 1986 AQCD (U.S. EPA, 1986a), several studies have shown seasonal variation in blood Pb concentrations in children (e.g., Havlena et al., 2009; Gulson et al., 2008; Kemp et al., 2007; Laidlaw et al., 2005; Haley and Talbot, 2004; Johnson and Bretsch, 2002; Yiin et al., 2000; Johnson et al., 1996), with elevated concentrations during the warm season and lower levels in the cold season. Seasonal dynamics of blood Pb concentrations in children appear to be caused at least in part by seasonal patterns in access of children to soils and soil properties (e.g., moisture content) that may contribute to seasonal variation in entrainment of soil and dust Pb into breathing zone air (Laidlaw et al., 2012; Laidlaw et al., 2005; Johnson and Bretsch, 2002). Seasonal variation in blood Pb concentrations occur, and summertime blood Pb concentrations demonstrated the strongest association with soil Pb concentrations (Johnson and Bretsch, 2002). Laidlaw et al. (2012) observed that air Pb in the $PM_{2.5}$ fraction and $PM_{2.5}$ attributed to soil were elevated in the warm season compared with the cold season. Yiin et al. (2000) also found that geometric mean for blood Pb, floor Pb loading and concentration were statistically significantly higher in the hot season compared with the cold season, although carpet dust Pb loading was statistically significantly higher in the cold season compared with the hot season. However, regression of blood Pb on floor and windowsill dust with and without adjustment for the hot, warm, and cool seasons showed no statistically significant effect of the seasons directly on blood Pb. Meteorological factors appear to contribute to blood Pb seasonality, and the tendency of children to play outside during the warm months may also allow for more contact with soil Pb. Laidlaw et al. (2005) analyzed the temporal relationships between child blood Pb concentrations and various atmospheric variables in three cities (Indianapolis, IN: 1999-2002; Syracuse, NY: 1994-1998; New Orleans, LA: 1998-2003). Blood Pb data was obtained from public health screening programs conducted in the three cities. Blood Pb samples were dominated by children <5 years of age and age distribution varied across the three cities. The temporal variation in blood Pb concentrations in each city was predicted by multivariate regression models that included the following significant variables: PM_{10}, wind speed, air temperature, and soil moisture; as well as dummy variables accounting for temporal displacement of the effects of each independent variable on blood Pb. Laidlaw et al. (2005) reported R^2 values for the regression models, but did not report the actual regression coefficients. The R^2 values were as follows: Indianapolis 0.87 ($p = 0.004$); Syracuse 0.61 ($p = 0.0012$); New Orleans 0.59 ($p < 0.00001$).

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

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

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

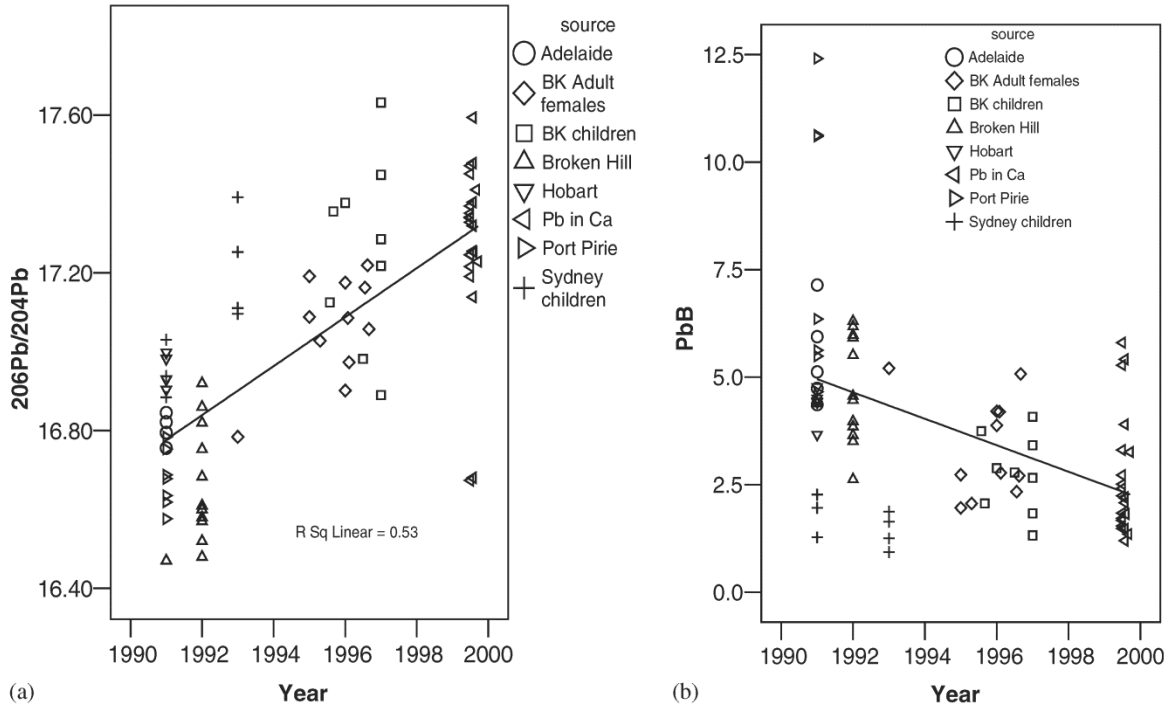
Pb body burden has been reported among individuals known to consume wild game hunted with Pb shot. For example, fifty men from Nuuk, Greenland participated in a study in which they recorded their diet and produced blood samples (Johansen et al., 2006). Men who regularly ate hunted sea birds had an average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

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

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

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

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



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

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

3.4.2 Pb in Bone

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

3.4.3 Pb in Urine

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

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

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

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

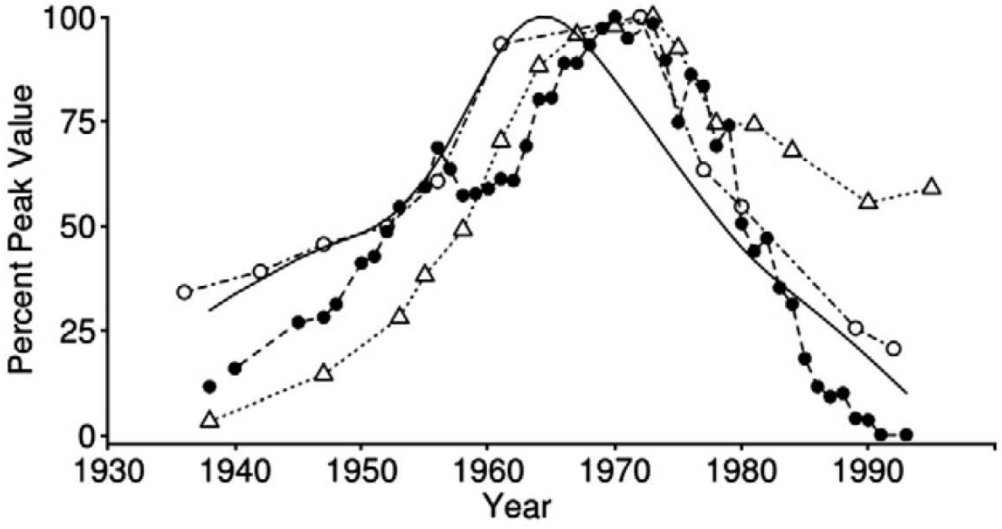
Survey Stratum	Period	Geometric Mean (µg Pb/g CR)[a]	95% Confidence Interval	Number of Subjects
	2003-2004	0.615	0.588, 0.644	1,281
	2005-2006	0.551	0.522, 0.582	1,271
	2007-2008	0.502	0.471, 0.534	1,327
Females	1999-2000	0.722	0.681, 0.765	1,238
	2001-2002	0.639	0.594, 0.688	1,355
	2003-2004	0.648	0.601,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

[a]Values are in µg Pb/g creatinine (CR)

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

3.4.4 Pb in Teeth

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



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

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

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

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

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

3.5 Empirical Models of Pb Exposure-Blood Pb Relationships

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

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

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

3.5.1 Air Pb-Blood Pb Relationships in Children

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

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

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

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

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

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

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

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

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

[b] ln(PbB) = ln(PbA) x 0.3485 + 2.853

[c] ln(PbB) = ln(PbA) x 0.2159 + 2.620

[d] ln(PbB) = ln(PbA) x 0.24 + 3.17

[e] PbB = PbA x 7.0, see Table 3-13 for more information.

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

[g] PbB = PbA x 8.6

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

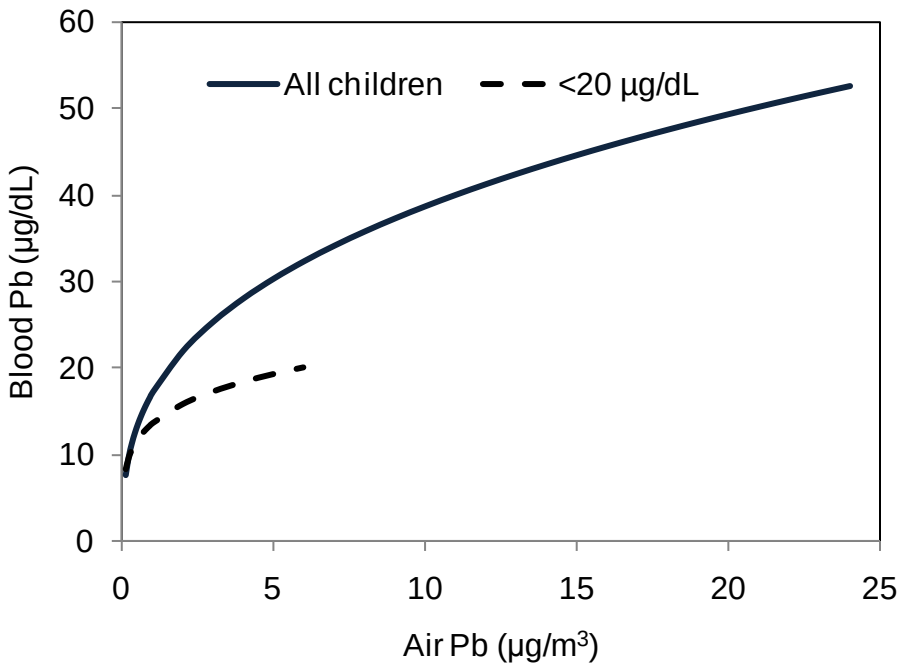
[i] PbB = Pb A x 3.6

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

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

[l] PbB = PbA x 9.63

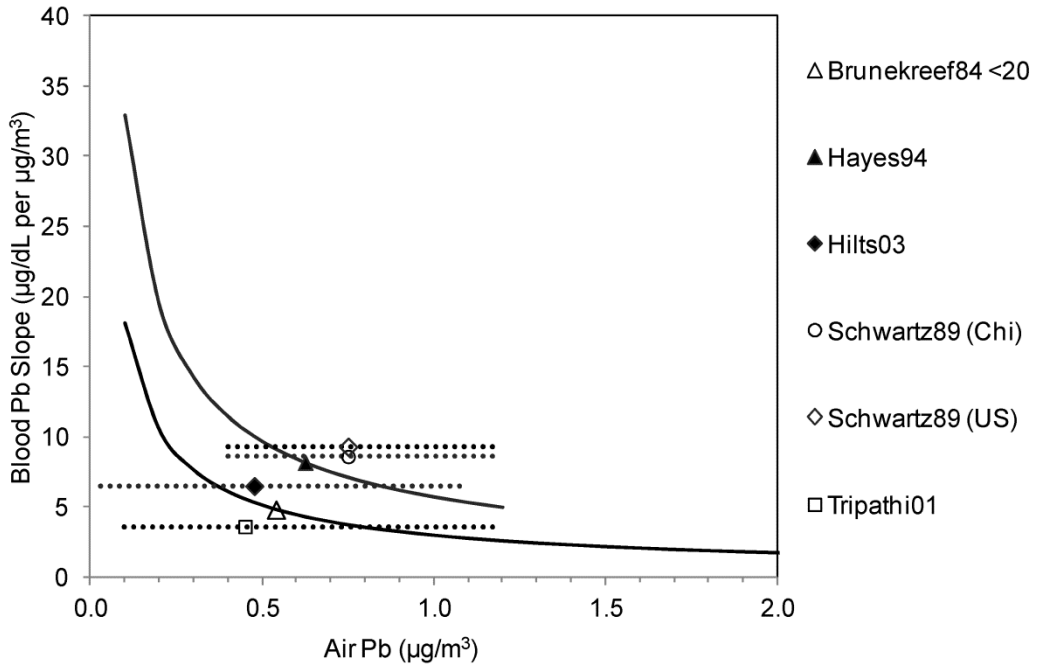
GM, geometric mean; GSD, geometric standard deviation; PbB, blood Pb concentration (µg/dL); PbA, air Pb concentration (µg/m^3)



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

Data provided from Brunekreef (1984).

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



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

Figure 3-23 Blood Pb – air Pb slopes (µg/dL per µg/m^3) predicted from epidemiologic studies.

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

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

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

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

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

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

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

[c] Values assumed by study authors.

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

Source: Data from Hilts (2003).

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

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

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

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

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

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

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

[a]Values are for 1990 and 1987 cohorts, respectively, at an air Pb concentration of 0.4 µg/m^3 which is the median and geometric mean of the annual air Pb concentrations over the course of the study based on data in Figure 1 of Schnaas et al. (2004)

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

For an approach that considers all the Schnaas et al. (2004) cohorts simultaneously, data for annual geometric mean of blood Pb and air Pb were extracted from Figure 1 in Schnaas et al. (2004). However, in employing this approach, blood Pb is confounded by age and year because in the early years of the study, only younger children were available and in the later years of the study, only older children contributed data. The extracted values of the geometric mean of blood Pb and mean air Pb were used in regressions employing linear and log-log models for comparison to other studies. The linear model obtained was: PbB = 2.50×PbA + 5.61 (R^2 = 0.84), i.e., the linear model produced a constant slope of 2.50 µg/dL per µg/m^3. However, inspection of the graph (not shown here) suggested a bi-linear fit. Regression of the data over the interval 0.1-0.4 µg/m^3 produced a slope of 9.0 µg/dL per µg/m^3 (R^2 = 0.83), and regression of the data over the interval 0.4-2.8 µg/m^3 produced a slope of 1.52 µg/dL per µg/m^3 (R^2 = 0.83). The log-log model was: ln(PbB) = 0.26×ln(PbA) + 2.20 (R^2 = 0.94), resulting in an inverse curve for dPbB/dPb, versus PbA, with a slope of 4.5 µg/dL per µg/m^3 at PbA = 0.4 µg/m^3.

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

Therefore, it is possible to infer an air Pb-blood Pb relationship from these data. Two sources of blood Pb data were used in Schwartz and Pitcher (1989): NHANES II provided measurements for U.S. individuals 6 months to 74 years of age (N = 9,987 subjects) between February 1976 and February 1980, and the Chicago blood Pb screening program provided measurements in black children aged birth to 5 years (N = 5476 subjects) for the period from 1976 to mid-1980. Observed blood Pb levels were not provided. Gasoline Pb consumption for the U.S. was estimated as the product of monthly gasoline sales and quarterly estimates of Pb concentrations in gasoline reported to U.S. EPA. Based on the NHANES blood Pb data for whites, the regression coefficient for blood Pb on the previous month's gasoline Pb usage, adjusted for age, race, sex, income, degree of urbanization, nutrient intake, smoking, alcohol consumption, occupational exposure, and other significant covariates was 2.14 µg/dL blood per 100 metric tons of gasoline Pb/day (SE=0.19, p=0.0000); the authors reported that the results for blacks were essentially identical. Based on the Chicago blood Pb data, the age-adjusted regression coefficient was 16.12 (µg/dL per 1,000 metric tons gasoline Pb/quarter [SE=1.37, p=0.0001]). When the coefficient was scaled by the ratio of Chicago's gasoline use to the nation's and converted to units of 100 metric tons per day, the gasoline Pb coefficient was 1.97 µg/dL blood per 100 metric tons of gasoline Pb/day), which is similar to the coefficient reported for the NHANES cohort. U.S. EPA (1986a) reported data on gasoline Pb consumption (sales) and ambient air Pb levels in the U.S. during the period 1976-1984 (Table 3-15). Based on these data, air Pb concentrations decreased in association with gasoline Pb consumption. The linear regression coefficient for the air Pb decrease was 0.23 $\mu g/m^3$ per 100 metric tons gasoline Pb/day (SE = 0.02, $R^2 = 0.95$, $p < 0.0001$). If this regression coefficient is used to convert the blood Pb slopes from Schwartz and Pitcher (1989), the corresponding air Pb-blood Pb slopes would be 9.3 and 8.6 µg/dL per $\mu g/m^3$, based on the NHANES and Chicago data, respectively (e.g., 2.14/0.23 = 9.3 and 1.97/0.23=8.6).

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

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

The linear regression coefficient is 0.23 µg/m^3 air per 100 metric tons/day (SE= 0.020, R^2 = 0.95, p <0.0001)

[a]Conversion factor is 10/365 days/year.

[b]Annual mean of per site maximum quarterly means (1984 Trend Report, Available online: http://www.epa.gov/air/airtrends/pdfs/Trends_Report_1984.pdf

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

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

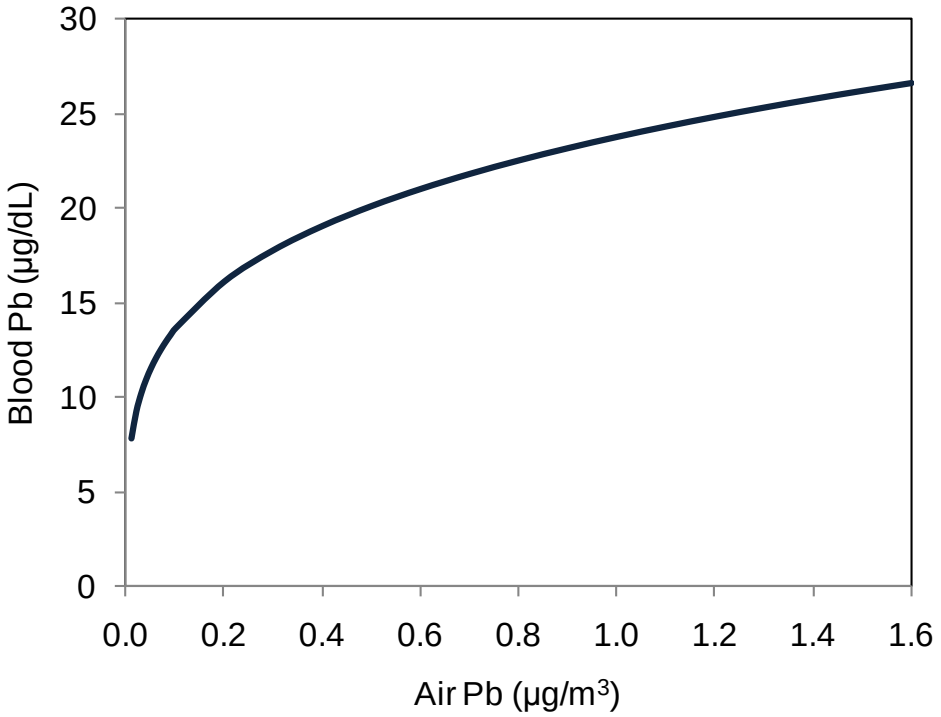
Table 3-16 Air Pb concentrations and blood Pb levels in children in Mumbai, India.

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

The linear regression coefficient is 3.62 µg/dL blood per µg/m^3 air (SE= 0.61, R^2 = 0.76, p <0.001).
GM, geometric mean; GSD, geometric standard deviation; N, number of subjects.

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

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



Note: The regression model is: (ln[µg/dL blood Pb] = 0.24·ln[µg/m^3 air Pb] + 3.17).
Modified from Hayes et al. (1994).

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

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

(2001) is consistent with this observation, given that the blood Pb levels were at the lower end of this range (i.e., 10-15 µg/dL).

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

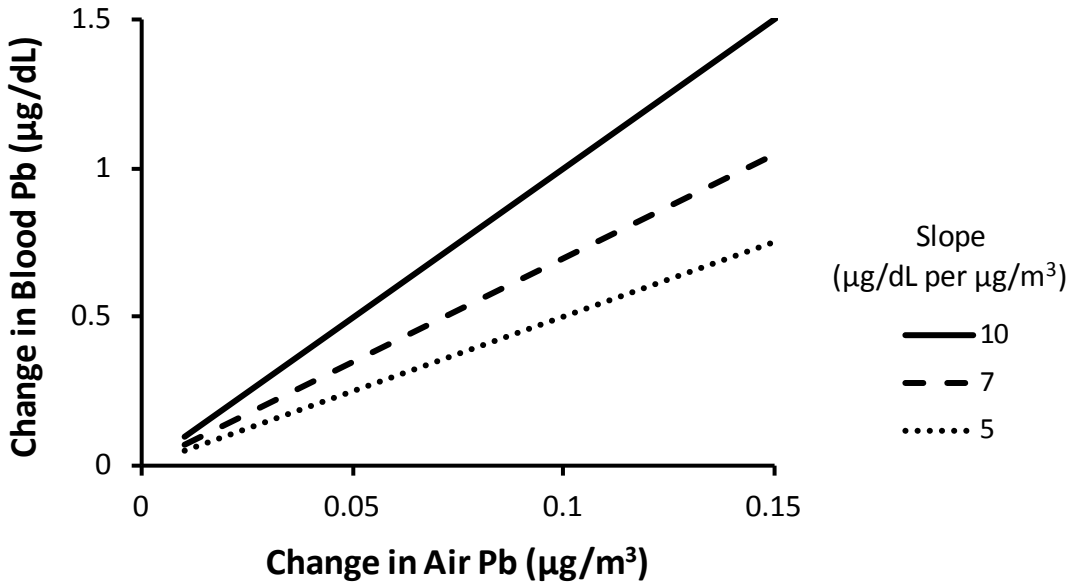


Figure 3-25 Effect of air-to-blood slope on estimated change in air-related blood Pb with change in air Pb.

3.5.2 Air Pb-Blood Pb Relationships in Occupational Cohorts

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

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

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

3.5.3 Environmental Pb-Blood Pb Relationships

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

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

Equation 3-2

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

Equation 3-3

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

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

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

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

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

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

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

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

[a]Children N = 2,155 (age 10-60 months); R^2 = 40%

[b]Includes the 50 states and the District of Columbia

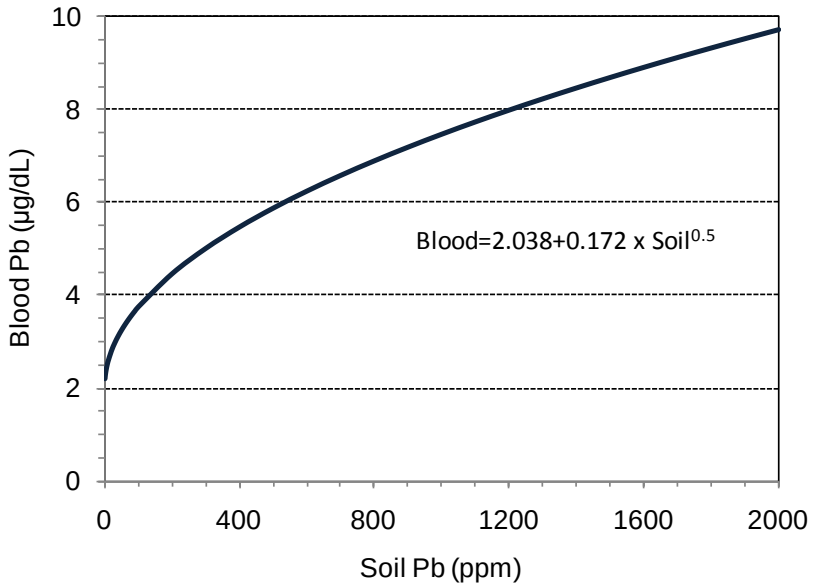
Source: Dixon et al. (2009).

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

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

Equation 3-4

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



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

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

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

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

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

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

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

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

R^2 = 0.264; p <0.0001; based on data from Bunker Hill Superfund Site, collected over the period 1988-2002.
GM: geometric mean; ln: natural log.
Source: TerraGraphics (2004).

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

3.6 Biokinetic Models of Pb Exposure-Blood Pb Relationships

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

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

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

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

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

3.7 Summary and Conclusions

3.7.1 Exposure

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

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

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

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

3.7.2 Toxicokinetics

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

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

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

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

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

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

3.7.3 Pb Biomarkers

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

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

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

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

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

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

3.7.4 Air Lead-Blood Lead Relationships

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

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CHAPTER 4 INTEGRATED HEALTH EFFECTS OF LEAD EXPOSURE

4.1 Introduction

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

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

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

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

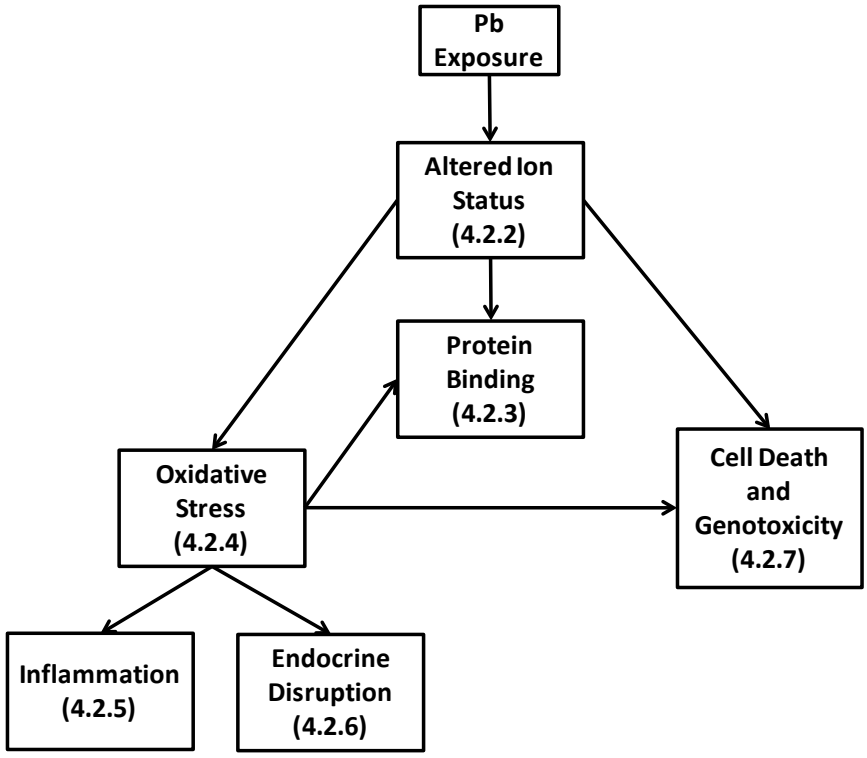
Judgments regarding causality also were made by evaluating the evidence for Pb exposure routes and the full range of concentrations in animal toxicological and epidemiologic studies considered relevant to this ISA, i.e., blood Pb levels up to 30 µg/dL for most outcomes as described in Section 1.1. Studies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

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

4.2 Modes of Action

4.2.1 Introduction

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



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

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

4.2.2 Altered Ion Status

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

4.2.2.1 Disruption of Ca^{2+} Homeostasis

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

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

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

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

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

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

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

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

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

4.2.2.2 Disruption of Ion Transport Mechanisms

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

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

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

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

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

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

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

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

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

Disruption of Neurotransmitter Release

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

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

Ca^{2+} mimic in regard to independently inducing exocytosis and evoking neurotransmitter release at higher concentrations (Lasley and Gilbert, 2002). Suszkiw (2004) reports that augmentation of spontaneous release of neurotransmitters may involve Pb-induced activation of CaMKII-dependent phosphorylation of synapsin I or direct activation of synaptotagmin I. Further, Suszkiw (2004) suggests that unlike the intracellularly mediated effects of Pb on spontaneous release of neurotransmitters, Pb-induced inhibition of evoked transmitter releases is largely due to extracellular blockage of the voltage-gated Ca^{2+} channels.

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

4.2.2.3 Displacement of Metal Ions and Perturbed Protein Function

The binding of metal ions to proteins causes specific changes in protein shape, and these conformational changes may alter specific cellular function of many proteins (Kirberger and Yang, 2008). Metal binding sites on proteins are generally ion-specific and are influenced by multiple factors, including binding geometries, ligand preferences, ionic radius, and metal coordination numbers (Kirberger and Yang, 2008; Garza et al., 2006). The coordination chemistry that normally regulates metal-protein binding makes many proteins particularly susceptible to perturbation from Pb, as it is able to function with flexible coordination numbers and can bind multiple ligands (Kirberger and Yang, 2008; Garza et al., 2006). However, due to differences in its physical properties, Pb induces abnormal conformational changes when it binds to proteins (Kirberger and Yang, 2008; Bitto et al., 2006; Garza et al., 2006; Magyar et al., 2005), and these structural changes elicit altered protein function. It is known that $[Ca^{2+}]_i$ is an important second messenger in cell signaling pathways, and operates by binding directly to and activating proteins such as calmodulin and PKC (Goldstein, 1993). Alterations in the functions of both of these proteins due to direct interaction with Pb have been well documented in the literature.

PKC is a family of serine/threonine protein kinases critical for cell signaling and important for cellular processes, including growth and differentiation (Goldstein, 1993). PKC contains a "C2" Ca^{2+}-binding domain and requires binding of the cation, as well as the presence of diacylglycerol and phospholipids, for proper cellular activity (Garza et

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

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

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

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

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

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

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

Zn-binding domains are also found in transcription factors and proteins necessary for gene expression, including GATA proteins and transcription factors TFIIIA, Sp1, and Erg-1 (Ghering et al., 2005; Huang et al., 2004; Reddy and Zawia, 2000; Hanas et al., 1999; Zawia et al., 1998). Pb was found to form tight complexes with the cysteine residues in GATA proteins (Pb stoichiometric stability constants of $[^{CF}\beta_1{}^{Pb}] = 6.4 (\pm 2.0) \times 10^9\ M^{-1}$ for single C-terminal GATA Zn finger from chicken and $^{DF}\beta_2{}^{Pb2} = 6.3 (\pm 6.3) \times 10^{19}\ M^{-2}$ for double-GATA Zn finger from human), and was able to displace bound Zn from the protein under physiologically relevant conditions (Ghering et al., 2005). Once Pb was bound to GATA proteins, they displayed decreased ability to bind to DNA (Pb concentrations ≥ 1.25 µM) and activate transcription. Pb at a minimum concentration of 10 µM also binds to the Zn domain of TFIIIA, inhibiting its ability to bind DNA (Huang et al., 2004; Hanas et al., 1999). Huang et al. (2004) also reported that exposure to Pb caused the dissociation of TFIIIA-DNA adducts and using NMR spectroscopy, found that altered TFIIIA activity was the result of a Pb-induced abnormal protein conformation.

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

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

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

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

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

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

4.2.2.4 Mitochondrial Abnormality

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

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

Altered Energy Metabolism

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

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

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

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

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

Altered Heme Synthesis

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

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

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

4.2.3 Protein Binding

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

4.2.3.1 Intranuclear and Cytoplasmic Inclusion Bodies

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

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

4.2.3.2 Cytosolic Pb Binding Proteins

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

cytosolic Pb-binding proteins have been identified in the kidneys of Pb-exposed rats and humans, including the cleavage product of α2-microglobulin, acyl-CoA binding protein (MW = 9 kDa), and thymosin β4 (MW = 5 kDa) (Smith et al., 1998; Fowler and DuVal, 1991).

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

4.2.3.3 Erythrocytic Pb Binding Proteins

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

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

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

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

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

4.2.3.4 Metallothionein

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

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

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

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

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

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

4.2.4 Oxidative Stress

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

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

4.2.4.1 δ-ALA Oxidation

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

4.2.4.2 Membrane and Lipid Peroxidation

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

hydrogen (H) removal easier (Yiin and Lin, 1995). After the essential unsaturated fatty acid solutions were incubated with Pb (4-12 µg/dL, 24 hours), the production of malondialdehyde (MDA), a marker of oxidative stress and lipid oxidation end product, increased relative to the number of double bonds of the fatty acid (Yiin and Lin, 1995). In the absence of Fe^{2+}, Pb has not been shown to promote lipid peroxidation; however, it may accelerate peroxidation by H_2O_2 (Quinlan et al., 1988). This could be due to altering membrane structure, restricting phospholipid movement, and facilitating the propagation of peroxidation.

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

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

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

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

4.2.4.3 NAD(P)H Oxidase Activation

NAD(P)H oxidase is a membrane bound enzyme that requires Ca^{2+} in order to catalyze the production of O_2^- from NAD(P)H and molecular oxygen (Leseney et al., 1999). Two studies provide evidence for increased activation of NAD(P)H oxidase that may contribute to the production of ROS after Pb exposure (Ni et al., 2004; Vaziri et al., 2003). Vaziri et al. (2003) found increased protein expression of the NAD(P)H subunit gp^{91}phox in the brain, heart, and renal cortex of Pb-treated rats (100 ppm in drinking water, 12 weeks). This upregulation was present in Pb-treated (1-10 ppm) human coronary artery endothelial cells, but not vascular smooth muscle cells (VSMC), which do not express the protein (Ni et al., 2004). It is possible that NAD(P)H oxidase serves as a potential source of ROS in cells that express this protein.

4.2.4.4 Antioxidant Enzyme Disruption

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

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

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

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

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

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

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

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

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

4.2.4.5 Nitric Oxide Signaling

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

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

Soluble Guanylate Synthase

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

4.2.5 Inflammation

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

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

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

Inflammation can be mediated by the production of chemical messengers such as prostaglandins (PG). Pb exposure has been associated with increased arachidonic acid (AA) metabolism, thus elevating the production of PGE_2, PGF_2, and thromboxane in occupationally-exposed humans (mean blood Pb level 48 µg/dL) (Cardenas et al., 1993) and animal and cell models (e.g., 0.01 µM, 48 hours) (Chetty et al., 2005; Flohé et al., 2002; Knowles and Donaldson, 1997; Lee and Battles, 1994). Dietary Pb exposure of animals (500 ppm, 19 days) can increase the percentage of cell membrane AA, the precursor of cyclooxygenase and lipoxygenase metabolism to PGs and leukotrienes (Knowles and Donaldson, 1990). Additionally, Pb (1 µM) may promote the release of AA via activation of phospholipase A_2, as shown in isolated VSMC (Dorman and Freeman, 2002).

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

4.2.5.1 Cytokine Production

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

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

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

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

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

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

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

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

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

4.2.6 Endocrine Disruption

4.2.6.1 Hypothalamic-Pituitary-Gonadal Axis

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

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

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

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

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

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

A number of studies have revealed that Pb exposure affects the dynamics of growth (see Section 4.8.1). Decreased growth after Pb exposure could be the result of Pb-induced decreased GH levels (Berry et al., 2002; Camoratto et al., 1993; Huseman et al., 1992; Huseman et al., 1987). This decrease in GH could be a result of decreased release of GH releasing hormone (GHRH) from the hypothalamus or disrupted GHRH binding to its receptor, which has been reported in vitro after Pb treatment (IC_{50} free Pb in solution 5.2 × 10^{-5} µM, 30 minutes) (Lau et al., 1991). GH secretion may also be altered from decreased testosterone resulting from Pb exposure.

4.2.6.2 Hypothalamic-Pituitary-Thyroid Axis

The evidence for the effects of Pb exposure on the hypothalamic-pituitary-thyroid (HPT) axis is mixed. Pb exposure impacts a variety of components in the thyroid hormone system. A number of occupational studies (blood Pb levels >7.3 µg/dL) have shown that elevated blood Pb is associated with lower thyroxine (T_4) (and free T_4 levels) without alteration in triiodothyronine (T_3), suggesting that long-term Pb exposure may depress thyroid function in workers (Dundar et al., 2006; Tuppurainen et al., 1988; Robins et al., 1983). However, animal studies on thyroid hormones have shown mixed results. Pb-exposed cows (blood Pb levels >51 µg/dL) were reported to have an increase in plasma T_3 and T_4 levels (Swarup et al., 2007), whereas mice and chickens manifested decreased serum T_3 concentrations after Pb exposure, which was accompanied by increased lipid peroxidation (Chaurasia et al., 1998; Chaurasia and Kar, 1997). Both decreased serum T_3 and increased lipid peroxidation were restored by vitamin E treatment, suggesting the disruption of thyroid hormone homeostasis could be a result of altered membrane architecture and oxidative stress; however, no data were provided to exclude changes in Pb kinetics as the mechanism of protection (Chaurasia and Kar, 1997).

Decreased T_4 and T_3 may be the result of altered pituitary release of thyroid stimulating hormone (TSH). However, several studies have reported higher TSH levels in high-level Pb-exposed workers (blood Pb levels >39 µg/dL) (Lopez et al., 2000; Singh et al., 2000; Gustafson et al., 1989), which would result in increased T_4 levels. Overall, results on the effects of Pb on the HPT axis are inconclusive.

4.2.7 Cell Death and Genotoxicity

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

4.2.7.1 DNA Damage

A number of studies in human populations have observed associations between indicators of Pb exposure and increased DNA damage, as measured as DNA strand breaks. Most of these associations have been observed in occupationally-exposed populations (Grover et al., 2010; Minozzo et al., 2010; Shaik and Jamil, 2009; Danadevi et al., 2003; Hengstler et al., 2003; Palus et al., 2003; Fracasso et al., 2002; de Restrepo et al., 2000). Evidence overall was equivocal in regard to how blood Pb levels correlated with DNA damage: Fracasso et al. (2002) observed that DNA damage increased with increasing blood Pb levels (blood Pb levels, <25, 25-35, and >35 µg/dL), whereas Palus et al. (2003) (mean blood Pb level: 50.4 µg/dL [range: 28.2 to 65.5 µg/dL]) and Minozzo et al. (2010) (mean [SD]: 59.43 µg/dL [28.34]) observed no correlation. Hengstler et al. (2003) examined workers exposed to Pb, Cd, and cobalt (Co) and observed that neither blood (mean: 4.4 [IQR: 2.84-13.6] µg/dL) nor air Pb levels (mean: 3.0 [IQR: 1.6-50.0] µg/m^3) were associated with DNA damage when examined alone, but that blood Pb influenced the occurrence of single strand DNA breaks when included in a multiple regression model along with Cd in air and blood and Co in air.

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

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

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

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

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

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

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

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

4.2.7.2 Mutagenicity

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

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

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

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

4.2.7.3 Clastogenicity

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

Sister Chromatid Exchange

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

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

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

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

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

Micronucleus Formation

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

Micronucleus formation in response to Pb exposure has been observed in rodent animal studies. Celik et al. (2005) observed that exposure of female rats to Pb acetate (140, 250, or 500 mg/kg once per week for 10 weeks) resulted in statistically significant increases in numbers of micronucleated polychromatic erythrocytes (PCEs) compared to controls. Similarly, Alghazal et al. (2008b) exposed rats to Pb acetate (100 ppm daily for 125 days) and observed statistically significant increases in micronucleated PCEs in both sexes. Tapisso et al. (2009) treated rats with Pb acetate (21.5 mg/kg; 1/10th the LD_{50}) via i.p. injection on alternating days for 11 or 21 days, for a total of 5 or 10 exposures. Formation of micronuclei in the bone marrow of exposed rats was increased over formation in controls in a significant duration-dependent manner. Two further studies investigated formation of micronuclei in the bone marrow of exposed mice: Roy et al. (1992) treated mice with Pb nitrate (10 or 20 mg/kg, i.p.) and observed a concentration-dependent increase in micronuclei, whereas Jagetia and Aruna (1998) observed an increase in micronuclei in mice treated with Pb nitrate (0.625-80 mg/kg, i.p.), though the increase was not concentration-dependent. Mice exposed to Pb acetate (0.1 µg/L via drinking water, a more environmentally relevant route of exposure, for 90 days) had statistically significant increases in micronucleated PCEs (Marques et al., 2006).

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

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

Chromosomal Aberrations

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

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

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

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

4.2.7.4 Epigenetic Effects

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

DNA Methylation

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

Mitogenesis

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

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

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

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

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

4.2.7.5 Gene Expression

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

Additional animal studies provide further evidence that exposure to Pb compounds can perturb gene expression. Zawia and Harry (1995) investigated whether the observed Pb-induced disruption of myelin formation in rat pups exposed postnatally was due to altered gene expression. In pups exposed to 2,000 ppm Pb acetate via lactation from PND1-PND20, the expression of proteolipid protein, a major structural constituent of myelin, was elevated (statistically significant) at PND20, compared to controls. The expression of another structural element of myelin (myelin basic protein) was similarly elevated in exposed animals, although not significantly so. The expression of both genes returned to control levels 5 days following the termination of exposure. These data suggest that altered gene expression in structural myelin proteins due to Pb exposure may be responsible for observed alterations in abnormal conduction of nerve impulses. Long et al. (2011) investigated the Pb-induced increase in ABCC5, an ATP-binding cassette transporter, in embryonic and adult zebrafish. In the initial in vitro portion of the study, exposure of zebrafish fibroblasts to 20 μM Pb nitrate for 24 hours significantly increased the induction of ABBC5 mRNA 2.68-fold over controls. Similar levels of induction were observed when embryonic zebrafish were exposed to 5 μM for 24 to 96 hours; specifically, induction of ABCC5 was seen in the livers of developing embryos. In adult fish, induction of ABCC5 was observed in the brains, intestines, and kidneys of exposed fish, but a decrease was found in their livers. Induction of ABCC5 in adult fish was observed to attenuate the toxicity of Cd (but not Hg or As); however, in developing embryos, the attenuation of Pb-induced toxicity was not investigated. These findings

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

4.2.7.6 Apoptosis

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

4.2.8 Summary

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

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

Mode of Action [Related Health Effects (ISA Section)]	Concentrations or Doses (Conditions)[a]	
	Blood Pb	**Dose**
Altered Ion Status [All Health Effects of Pb, Chapter 4]	3.5 µg/dL (Mean in cord blood; association with cord blood Ca^{2+}ATPase pump activity) Huel et al. (2008)	0.00005 µM free Pb^{2+} (In vitro; 30 minutes; calmodulin activation assay) Kern et al. (2000)
Protein Binding [Renal (4.5), Hematological Effects (4.7)]	17.0 µg/dL (Concurrent mean in adult workers with wildtype metallothionein expression; increased BP susceptibility) Chen et al. (2010a)	50 µM Pb glutamate (In vitro; 24 hours; increased nuclear protein in neurological cell) Klann and Shelton (1989)
Oxidative Stress [All Heath Effects of Pb, Chapter 4]	5.4 µg/dL (Concurrent mean in adult male workers; decreased CAT activity in blood) Conterato et al. (2013)	0.1 µM Pb acetate (In vitro; 48 hours; decreased cellular GSH in neuroblastoma cells) Chetty et al. (2005)
Inflammation [Nervous System (4.3), Cardiovascular (4.4), Renal (4.5), Immune (4.6), Respiratory (4.9.6), Hepatic (4.9.1)]	Among males with concurrent blood Pb ≥ 2.5 µg/dL (Increased serum TNF-α and blood WBC count) Kim et al. (2007)	0.01 µM Pb acetate (In vitro; 48 hours; increased cellular PGE_2 in neuroblastoma cells) Chetty et al. (2005)
Endocrine Disruption [Reproductive and Developmental Effects (4.8), Endocrine System (4.9.3), Bone and Teeth (4.9.4)]	1.7 µg/dL (Lowest level at which a relationship could be detected in adult women with both ovaries removed; increased serum FSH) Krieg (2007)	10 µM Pb nitrate (In vitro; 30 minutes; displaced GHRH binding to rat pituitary receptors) Lau et al. (1991)
Cell Death/Genotoxicity [Cancer (4.10), Reproductive and Developmental Effects (4.8), Bone and Teeth (4.9.4)]	3.3 µg/dL (Concurrent median in adult women; increased rate of HPRT mutation frequency) Van Larebeke et al. (2004)	0.03 µM Pb acetate (In vitro; 18 hours; increased formation of micronuclei) Bonacker et al. (2005)

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

The alteration of cellular ion status (including disruption of Ca^{2+} homeostasis, altered ion transport mechanisms, and perturbed protein function through displacement of metal cofactors) appears to be the major unifying mode of action underlying all subsequent modes of action (Figure 4-1). Pb is well characterized to interfere with endogenous Ca^{2+}

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

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

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

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

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

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

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

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

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

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

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

4.3 Nervous System Effects

4.3.1 Introduction

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

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

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

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

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

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

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

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

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

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

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

4.3.2 Cognitive Function

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

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

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

4.3.2.1 Full Scale IQ in Children

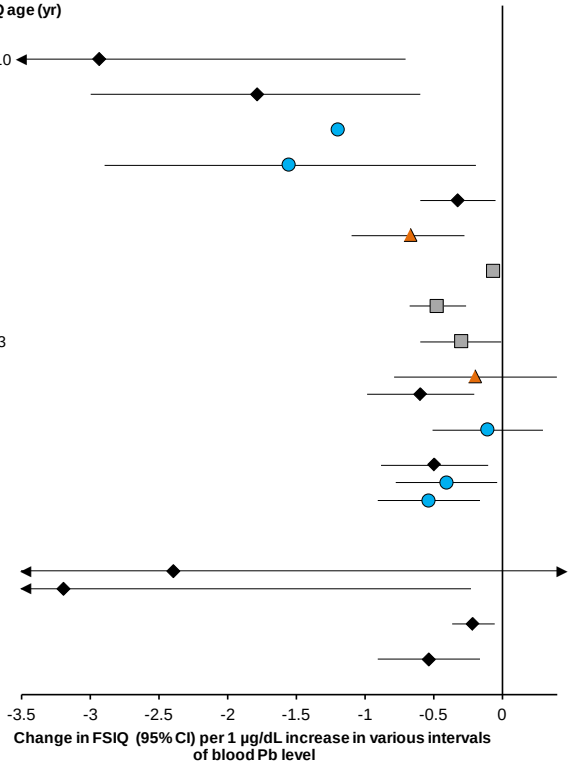
Evidence from Prospective Studies

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

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

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

Study	Blood Pb Metric Analyzed	Blood Pb Mean (SD) (µg/dL)	Blood Pb interval examined[a] (µg/dL)	FSIQ age (yr)
Prospective Studies				
Lanphear et al. (2005)	Concurrent, peak < 7.5	3.2	1.3-6	4.8-10
Canfield et al. (2003)	Concurrent, peak < 10	3.3	0.5-8.4	5
Jusko et al. (2008)[b]	Peak	11.4 (7.3)	2.1-10	6
Bellinger et al. (1992)	Age 2 yr, peak < 10	3.8	1-9.3	10
Dietrich et al. (1993)	Concurrent	11.8 (6.3) (Age 5)	5.5-10	6.5
Schnaas et al. (2006)	Prenatal (maternal)	7.8 (geometric)	3.2-10	6-10
Cooney et al. (1991)[b]	Age 3-5 yr avg	8.3 (Age 5 yr)	Not reported	7
Wasserman et al. (1997)	Age 0 to 7 yr avg	16.2 (geometric)	6.0-10	7
Tong et al. (1996)	Age 0 to 11-13 yr avg	14.0 (1.2) geometric	11-19	11-13
Kordas et al. (2011)	Prenatal (cord) Concurrent	6.6 (3.3) 8.1 (3.6)	3.2-11 4.3-10	4 4
Greene et al. (1992)	Age 2 yr	15.6 (1.4) geometric	10-24	4.8
Min et al. (2009)	Concurrent Age 4 yr Age 4 yr	7.0 (4.1)	3.0-10	4 9 11
Cross-sectional Studies				
Kim et al. (2009)	Concurrent, low Mn Concurrent, high Mn	1.73 (0.80)	0.9-2.8	8-11
Fulton et al. (1987)	Concurrent	11.5 (range: 3.3-34)	5.6-10	6-9
Roy et al. (2011)	Concurrent	11.5 (5.3)	5.9-10	3-7



[a]See Table 4-3 for explanation of the blood Pb level interval examined. Effect estimates were calculated for the lowest range examined in the study or the 10th percentile of blood Pb level to a blood Pb level of 10 µg/dL.
[b]Sufficient data were not available to calculate 95% CI.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

[c]Sufficient data were not available to calculate 95% CI.

[d]Results not included in Figure 4-2 because FSIQ assessed in adults.

[e]Results not included in Figure 4-2 because blood Pb level analyzed as categorical variable or because standardized regression coefficient reported.

[f]95% CIs were constructed using a standard error that was estimated for the reported p-value of 0.01.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Evidence from Cross-sectional Studies

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

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

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

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

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

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

Summary of Evidence for FSIQ

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

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

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

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

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

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

4.3.2.2 Bayley Scales of Infant Development

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

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

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

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

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

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

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

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

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

MDI = Mental Development Index, MTHFR = methylenetetrahydrofolate reductase

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

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

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

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

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

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

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

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

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

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

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

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

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

4.3.2.3 Learning and Memory in Children

Epidemiologic Studies of Learning and Memory in Children

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

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

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

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

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

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

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

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

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

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

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

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

[c]The direction of the effect estimate was changed such that a negative estimate represents poorer performance and a positive estimate represents better performance.

[d]Sufficient data were not available to calculate 95% CI.

[e]Effect estimate compares test performance of children in higher blood Pb groups to children in lowest blood Pb group.

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

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

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

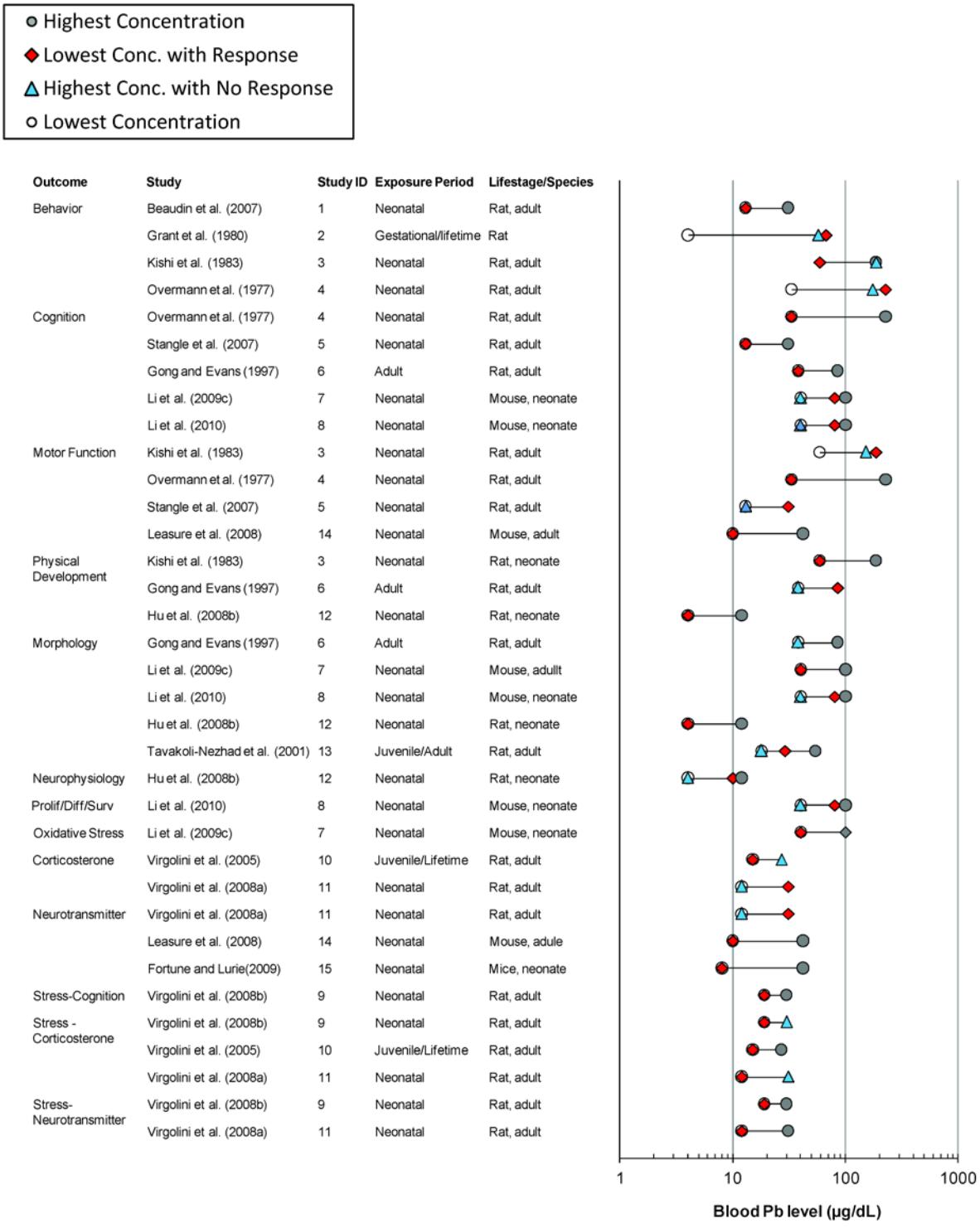
With respect to learning, associations in the Boston cohort were inconsistent across the various tests conducted, ages at which learning was assessed (ages 5, 10 years) and time periods of blood Pb levels (ages 1, 2, 5, 10 years) examined (Stiles and Bellinger, 1993; Bellinger et al., 1991). In two different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

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

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

Toxicological Studies of Learning and Memory

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



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

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

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

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

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

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

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

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

Learning and Memory – Morris Water Maze

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

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

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

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

Working Memory – Delayed Spatial Alternation

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

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

Learning and Memory - Y Maze

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

Learning - Schedule-Controlled Behavior Testing

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

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

Learning Ability with Stress

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

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

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

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

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

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

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

[c]Data from current study, Rossi-George et al. (2011)

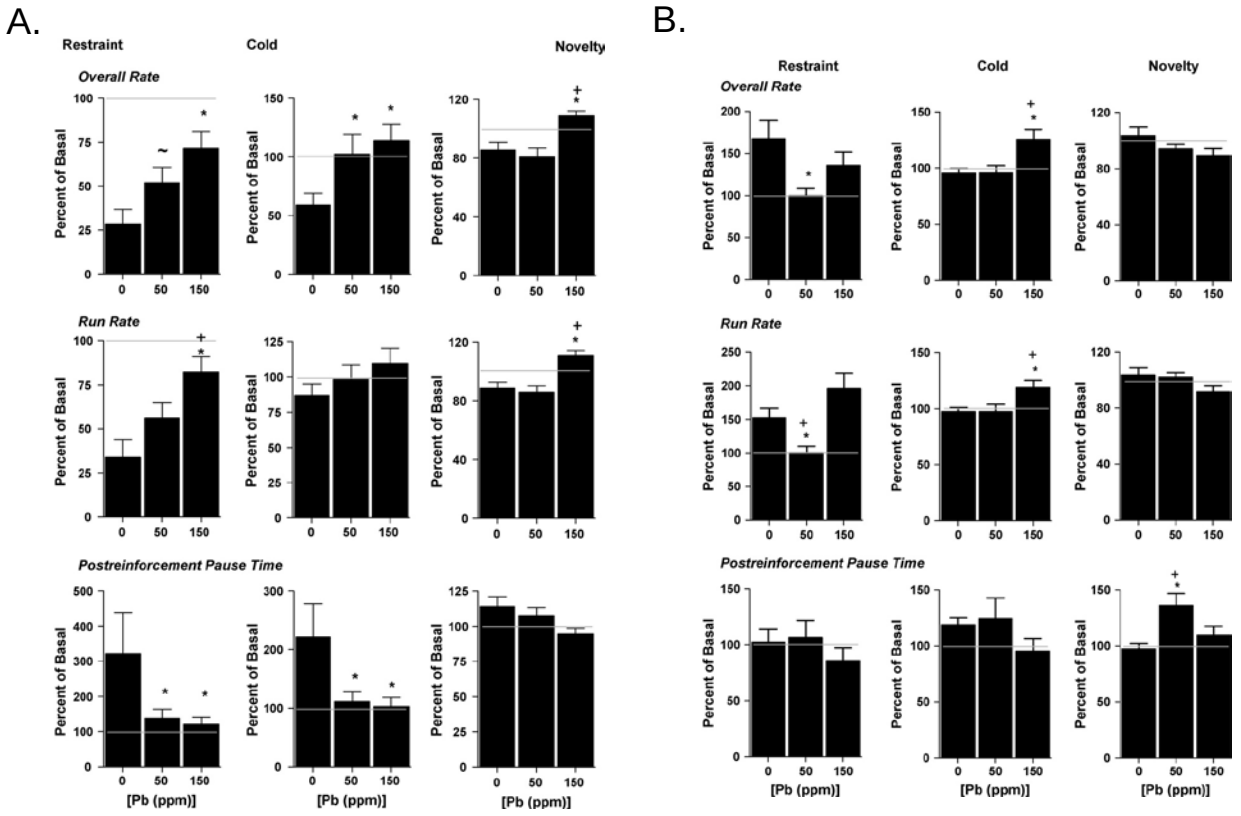
Notes: PRP = Post-reinforcement pause; PS = Prenatal (maternal stress); OS = Offspring stress.
*Denotes significant effect versus 0 ppm Pb, 0 stress control ($p < 0.05$).

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

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

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

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



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

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

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

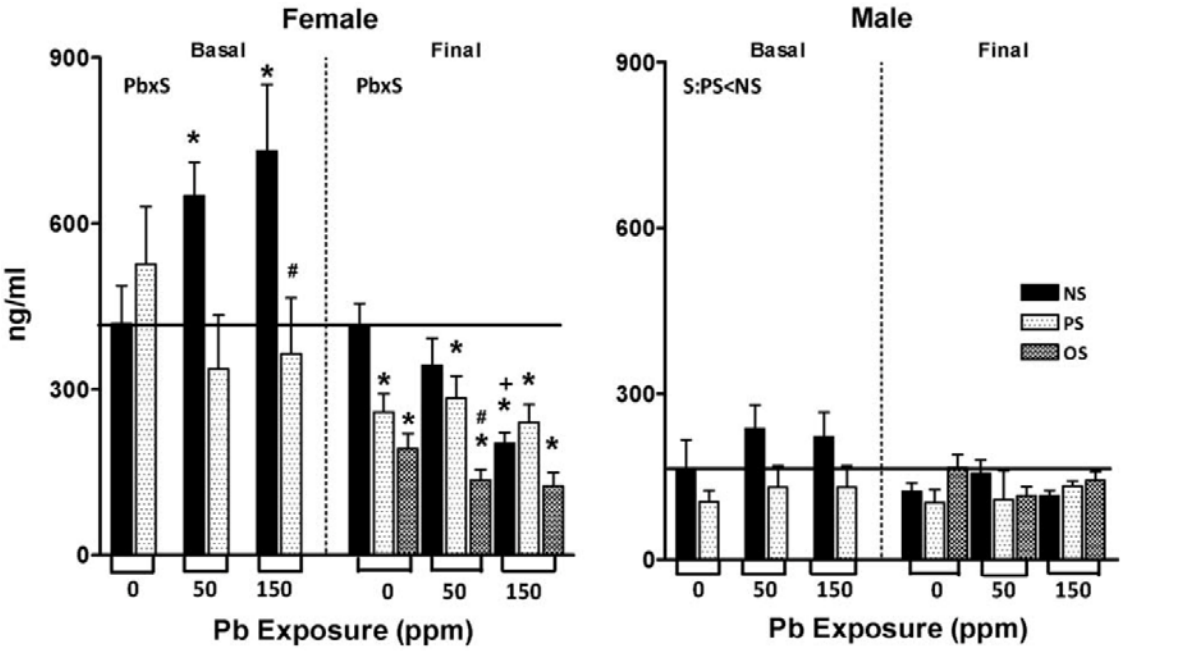
Pb exposure over various developmental windows in rodents has been shown to affect the HPA axis, as measured by the levels of corticosterone, the major glucocorticoid involved in stress responses in rodents. Thus, modulation of corticosterone may provide a

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

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

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

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



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

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

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

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

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

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

4.3.2.4 Executive Function in Children

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

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

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

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

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

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

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

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

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

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

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

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

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

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

[c]The direction of the effect estimate was changed such that a negative estimate represents poorer performance.

[d]95% CI was constructed using a standard error that was estimated from the reported p-value.

[e]Effect estimates compare test performance of children in higher blood Pb groups to children in lowest blood Pb group.

[f]Sufficient data were not available to calculate 95% CI.

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

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

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

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

4.3.2.5 Academic Performance and Achievement in Children

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

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

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

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

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

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

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

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

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

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

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

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

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

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

[c]Effect estimates compare test performance of children in higher blood Pb groups to children in lowest blood Pb group.

[d]95% CI was constructed using a standard error that was estimated from the reported p-value.

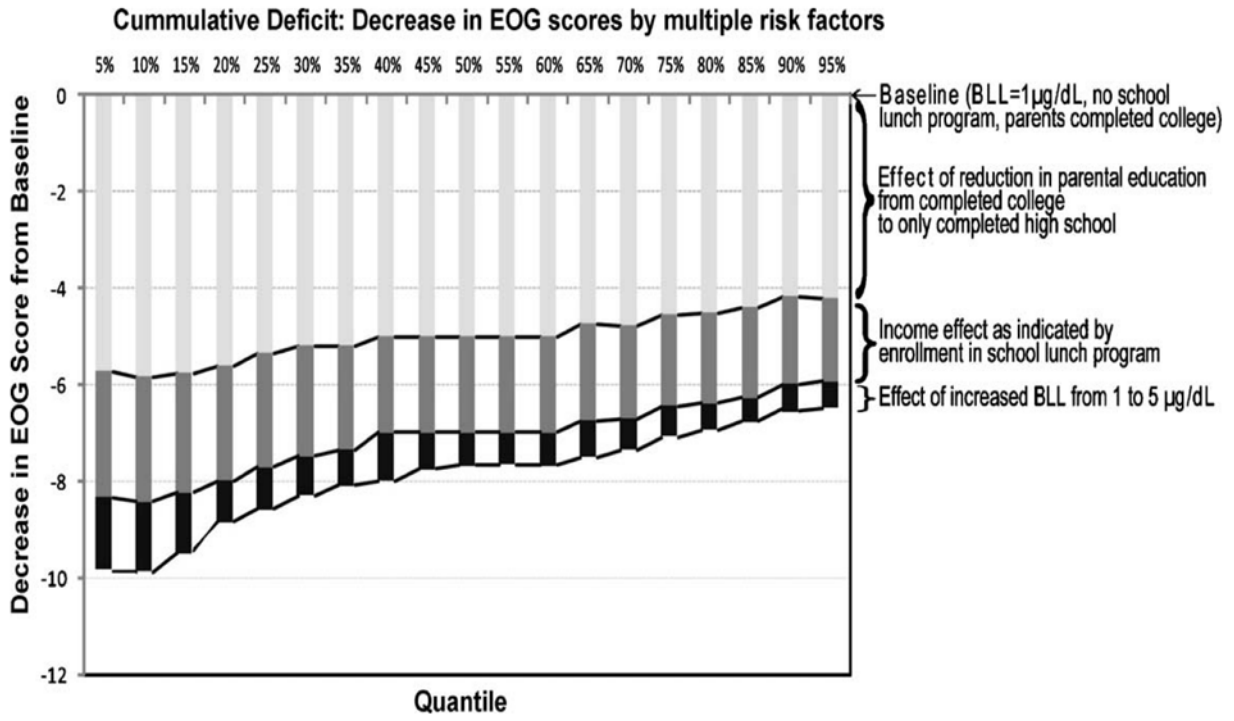
[e]Sufficient data were not available to calculate 95% CI.

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

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

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

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



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

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

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

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

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

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

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

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

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

4.3.2.6 Integrated Summary of Cognitive Function in Children

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

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

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

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

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

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

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

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

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

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

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

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

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

4.3.2.7 Epidemiologic Studies of Cognitive Function in Adults

Adults without Occupational Pb Exposures

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

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

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

Evidence from Prospective Studies

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

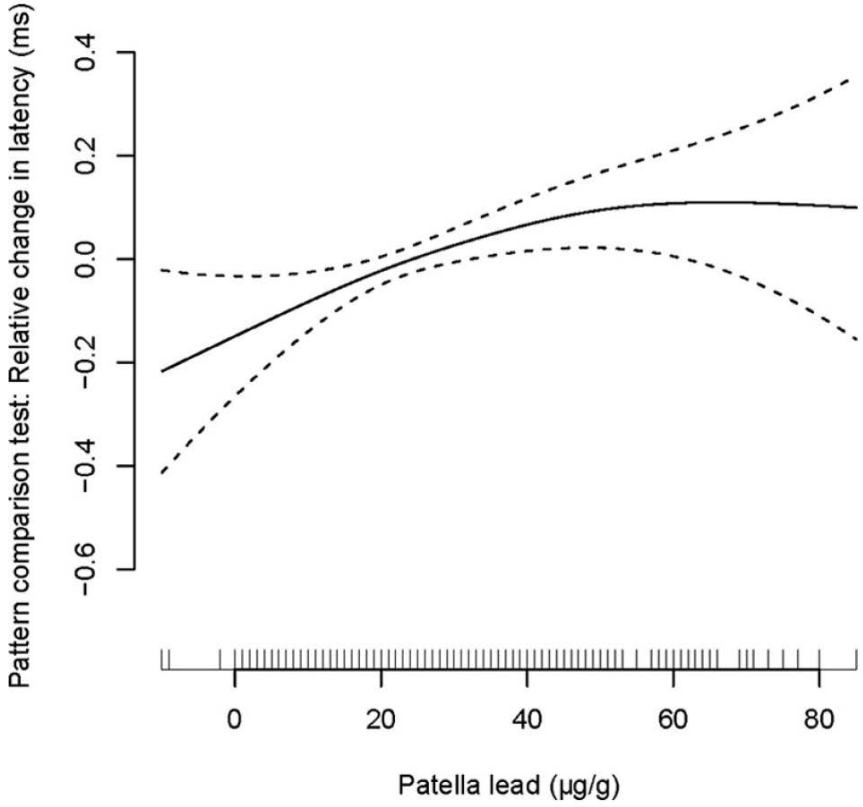
[a]Studies are presented first for prospective analyses then for cross sectional analyses. Within categories, results are grouped by representativeness of the population characteristics and blood Pb levels examined and extent of consideration for potential confounding. There is not necessarily a continuum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

[b]Effect estimates are for interactions between Pb and category of NPH, with the lowest tertile of NPH (fewer NPH) serving as the reference group.

[c]Effect estimates are for interactions between Pb and ALAD genotype.

[d]The directions of effect estimates were changed to indicate a negative slope as a decrease in cognitive performance.

[e]Results refer to mean cognitive test scores among tertiles of bone Pb. Tertile concentrations not reported.



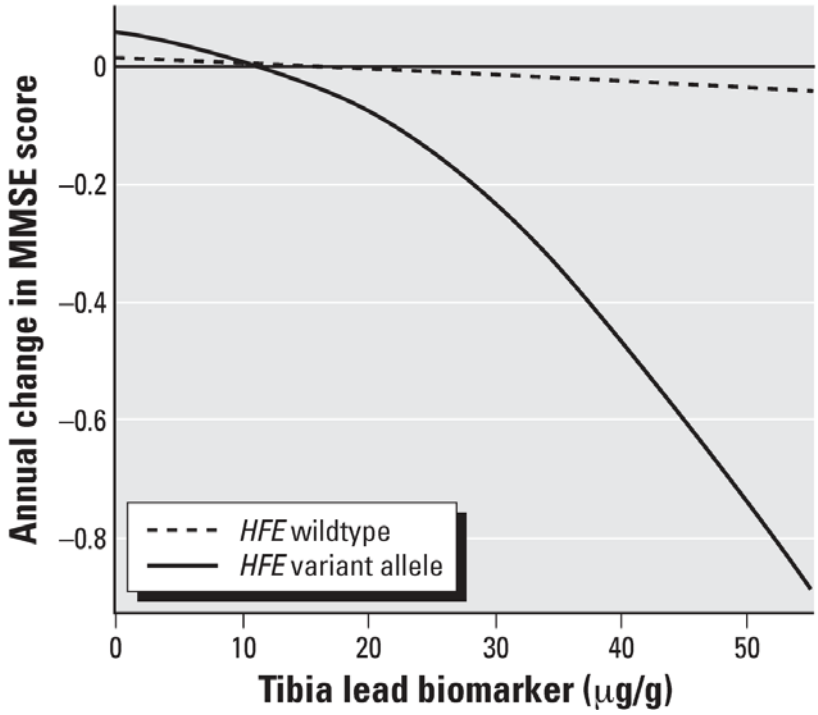
Note: Models are adjusted for age, age squared, education, smoking, alcohol intake, years between bone Pb measurement and first cognitive test, and years between the cognitive tests. The 9 subjects with the highest patella Pb levels (>89 µg/g bone mineral) were removed. The estimated concentration-response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

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

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

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

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



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

Source: Wang et al. (2007a).

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

Evidence from Cross-sectional Studies

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

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

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

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

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

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

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

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

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

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

Adults with Occupational Pb Exposures

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

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

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

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

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

Summary of Cognitive Function in Adults

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

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

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

Because bone Pb is a major contributor to blood Pb levels, blood Pb level also can reflect longer term exposures, including higher past exposures, especially in adults without

occupational exposures. Thus, in the NHANES results, it is difficult to characterize the relative contributions of recent and past Pb exposures to the associations observed between concurrent blood Pb level and cognitive function. In other cohorts, the discrepant findings for blood and bone Pb levels indicate that other indicators of cumulative Pb exposure (that likely included higher past exposures), may be better predictors of cognitive function in adults than is concurrent blood Pb level. Additional support for the effects of cumulative or past Pb exposure is provided by the analyses of a few child cohort as adults, which indicated that higher childhood tooth (from 1st/2nd grade) and blood (e.g., average to age 10 years) Pb levels were associated with decrements in cognitive function in adults ages 19-30 years (Mazumdar et al., 2011; Bellinger et al., 1994a). An uncertainty in the evidence for bone Pb-associated decrements in cognitive function is the potential residual confounding by age. Although studies adjusted for age and/or age-squared, the high correlation between increasing age and bone Pb levels (Section 3.3.5) makes it difficult to distinguish the independent effect of Pb exposure. However, the coherence with evidence for cognitive function decrements associated with long-term Pb exposure in animals provides support for associations observed in human adults.

Cross-sectional analyses provided information on potential effect modification of bone Pb- and blood Pb-associated decrements in cognitive function in adults by race, psychosocial stress, and genetic variants. Inconsistencies were found for effect modification by race in the BMS, ALAD genotype in the NAS and NHANES, and VDR genotype in NHANES. Larger tibia Pb-associated decrements in cognitive function were found in NAS men with HFE variants and in BMS subjects living near more psychosocial hazards. Evidence does not clearly indicate whether the observed effect modification reflects chance, a change in the toxicokinetics of Pb that alters Pb dose at the biological site of action, or a direct biological interaction that increases the toxicity of Pb in the target tissue. However, such effect modification serves to strengthen inferences about associations between Pb biomarkers and cognitive function since it is unlikely that potential confounding factors vary by levels of the modifying factor, particularly genetic variants. However, because there is little available evidence and inconsistent evidence for some factors, firm conclusions regarding effect modification are not warranted.

In contrast with nonoccupationally-exposed adults, in adults with former or current occupational Pb exposures, cognitive function decrements were associated with both blood (means: 31-42 μg/dL) and bone Pb levels. Thus, among Pb-exposed workers, both current and cumulative Pb exposures may affect cognitive function. Several of these studies considered confounding by a similar set of factors as studies of nonoccupationally-exposed adults but did not consider other occupational exposures that have been linked with cognitive function decrements including Mn. In a prospective

study of former Pb workers, peak tibia Pb levels were associated more strongly with cognitive performance than were concurrent blood Pb levels (Khalil et al., 2009a). These results also indicate that in the absence of high current Pb exposures, cumulative Pb exposures may have a greater effect on cognitive function in adults.

4.3.3 Externalizing Behaviors in Children

The effects of Pb exposure on externalizing behaviors such as attention, impulsivity, hyperactivity, destructive behavior, and truancy have not been examined as extensively as effects on cognition. Similar to cognitive function, several domains of externalizing behaviors are inter-related, making it difficult to examine whether Pb exposure has an effect on a particular domain. Externalizing behaviors are more complex to study than are cognitive effects, particularly FSIQ. There are fewer objective tests of externalizing behaviors with as strong psychometric properties or as rigorous validation as IQ tests. Externalizing behaviors often are assessed using teacher and/or parent ratings which are subject to greater measurement error. However, domain-specific neuropsychological assessments are advantageous as they may provide greater insight into whether there is a particular domain more susceptible to the effects of Pb exposure.

There are three major domains of externalizing behavior disorders: attention deficit hyperactivity disorder, undersocialized aggressive conduct disorder, and socialized aggressive conduct disorder (as reviewed inWhitcomb and Merrell, 2012). Characteristics of the attention deficit hyperactivity disorder domain include but are not limited to short attention span, distractibility, impulsivity, and hyperactivity. The undersocialized aggressive conduct disorder domain can be characterized by destructive, domineering / threatening or disruptive behaviors, and irritability and explosiveness. Behaviors included within the socialized aggressive conduct disorder domain include truancy from home or school, lying and cheating, and gang membership. In the sections that follow, to the extent possible, the evaluation of evidence for externalizing behaviors is organized by the domains described above then by specific behaviors included within a domain. Although not described explicitly as a part of either domain of conduct disorders, conduct disorders can be predictors of subsequent delinquency and criminality (Soderstrom et al., 2004; Babinski et al., 1999; Pajer, 1998). Therefore, evidence for criminal offenses is reviewed with conduct disorders.

As with cognitive function, in the evaluation of epidemiologic evidence for externalizing behaviors, greater emphasis was placed on evidence from more rigorous neuro-psychological tests, prospective studies with repeated assessments of blood Pb levels and behavior, studies of older children in whom outcomes are more reliably measured, and

studies of children whose blood Pb levels are less influenced by higher past Pb exposures. Similar to cognitive function, associations between Pb biomarker levels and externalizing behaviors potentially may be confounded by factors such as parental SES, education, and IQ, as well as quality and stability of the caregiving environment (often evaluated as HOME scoreTotsika and Sylva, 2004) and nutritional status. Accordingly, greater weight was given to studies with greater consideration for potential confounding in the study design or in statistical analyses. Consideration also was given to studies assessing effects relevant to blood Pb levels in current U.S. children (i.e., <5 µg/dL).

Externalizing behaviors and related clinically diagnosed disorders such as Attention Deficit Hyperactivity Disorder (ADHD) are considered to have a strong familial component (Whitcomb and Merrell, 2012; Dick et al., 2011; Faraone and Doyle, 2001; Kazdin, 1995; Campbell and Werry, 1986). In recent commentaries to studies reporting associations between blood Pb level and ADHD in children, Brondum (2011, 2007) asserted the need for studies to consider confounding by parental history of ADHD. Parental psychopathology potentially can affect associations between Pb and externalizing behaviors in the child through various theoretical mechanisms. One, if related only to the outcome and not to child Pb exposure, parental psychopathology can be a modifier of the child Pb exposure-externalizing behavior relationship. In this case, because the familial component of psychopathology explains a large portion of the variance in child behavior, not removing that variance with statistical adjustment could mask the smaller magnitude of risk due to other factors, including Pb. Thus, if there is an association between Pb and externalizing behaviors, it may be detected in an epidemiologic study by adjusting or stratifying by parental psychopathology. Characterization of effect modification by parental psychopathology also would depend on whether parental psychopathology is measured adequately. In the studies of Pb and externalizing behaviors, few studies have examined parental psychopathology, and none examined its role as a potential effect modifier. These studies measured parental psychopathology mostly as parental self report of ever having any among a list of many psychological, mental health, or psychiatric problems (Cho et al., 2010; Nicolescu et al., 2010; Silva et al., 1988) which is subject to recall error and lacks specificity for the condition being examined in the study (e.g., attention, hyperactivity, ADHD). Only Wang et al. (2008d) adjusted child ADHD associations for parent or sibling history of ADHD diagnosis, as ascertained from clinical records. Thus, in the few available studies, parental psychopathology and the familial component of externalizing behaviors likely was measured with error. However, in a potential role in effect modification, failure to account for parental psychopathology accurately or at all may attenuate or mask an association with Pb exposure if one exists.

Another way parental psychopathology potentially can influence a relationship between child Pb exposure and externalizing behaviors is by producing confounding bias. In this hypothetical case, parental psychopathology could produce an artifactual association between child Pb exposure and externalizing behaviors where there is no true association. However, this confounding bias would require that parental psychopathology not only be causally related to the outcome but also be correlated with child Pb exposure. One may speculate that parental psychopathology could affect child Pb exposure indirectly through parental caregiving quality. Parental caregiving quality is linked with externalizing behaviors in children (Nuttall et al., 2012; Campbell, 1995; Rothbaum and Weisz, 1994; Jacobvitz and Sroufe, 1987). Theoretically, parental psychopathology may result in poorer caregiving quality or neglect which may increase a child's exposure to Pb. There is not evidence describing this link. Cho et al. (2010) found similar concurrent blood Pb levels in children with and without parental history of psychopathology, but parental report of psychopathology is subject to measurement error. Further, if parental psychopathology is a determinant of Pb exposure, then inclusion as a model covariate would adjust for Pb exposure itself and underestimate a potential association with blood Pb. Studies that examined parenting behaviors in parents with current ADHD have indicated that parents with ADHD show negative parenting control, i.e., over-reactive disciplining, lack of planning, and disorganization but have not consistently indicated that parents with ADHD have poorer emotional responsiveness, i.e., involvement with the child (as reviewed in Johnston et al., 2012). Thus, it has not been demonstrated that parents with psychopathology have poorer caregiving quality or are neglectful. Nonetheless, if caregiving quality is the mechanism by which parental psychopathology potentially can confound associations between child Pb exposure and externalizing behaviors (because of a noncausal correlation between Pb exposure and parental psychopathology), then direct adjustment for parental caregiving quality would help address potential confounding by parental psychopathology. There still is the possibility of residual confounding by parental caregiving quality if not measured accurately, but this applies to any potential confounding factor examined for any outcome in relation to any exposure. Research has not established a direct relationship between parental psychopathology and child Pb exposure or one between parental psychopathology and poorer parental caregiving quality. Thus, based on the currently available evidence, in this ISA, parental psychopathology itself is not considered to be a potential confounder of associations between child Pb and externalizing behaviors. Instead, the ISA evaluates potential confounding by parental caregiving quality in addition to other factors well documented in the literature to be correlated with both Pb exposure and externalizing behaviors as described earlier in this section.

4.3.3.1 Attention, Impulsivity, and Hyperactivity in Children

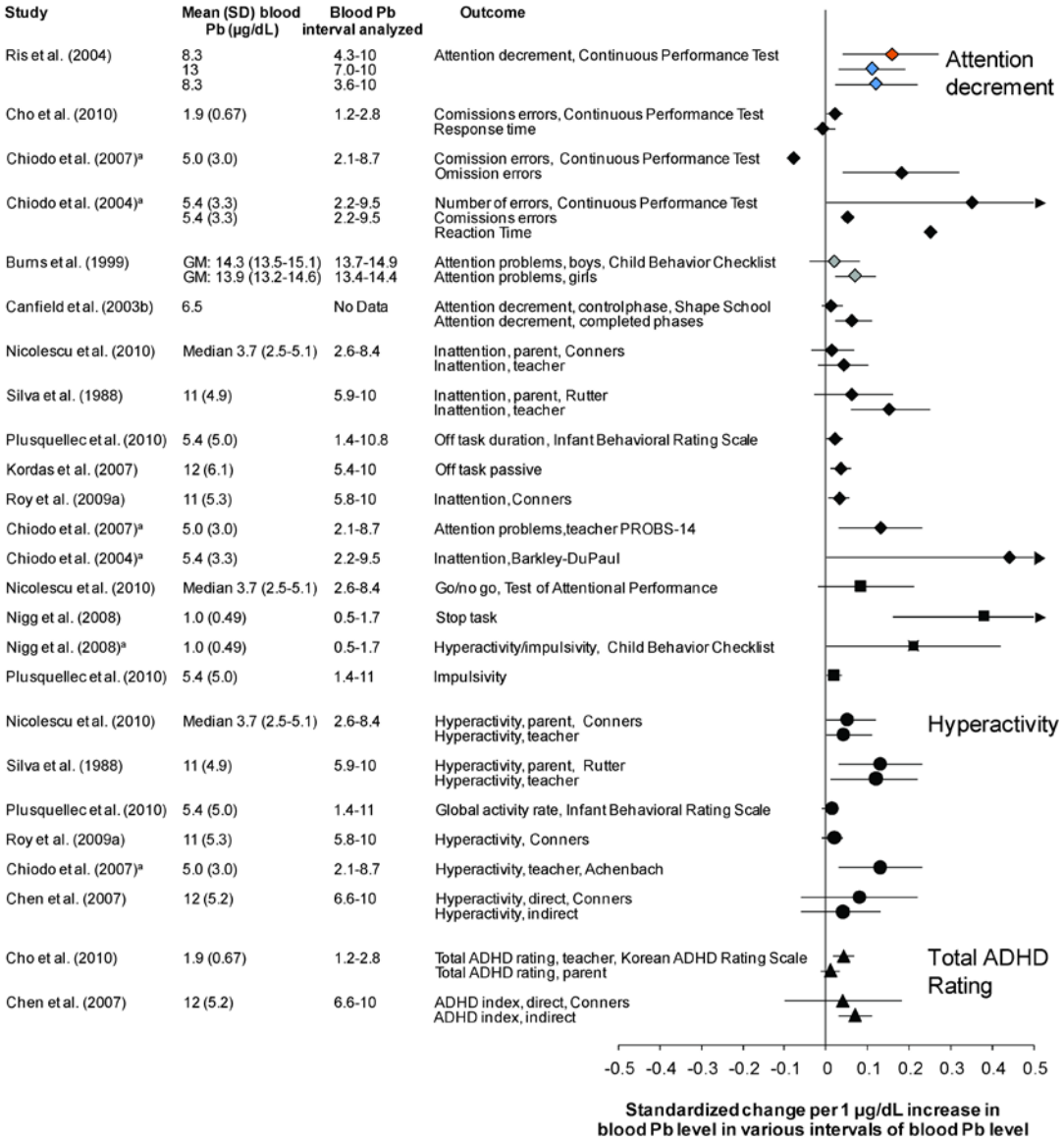
Within the attention deficit hyperactivity disorder domain of externalizing behaviors, studies of Pb exposure have focused primarily on attention, impulsivity, and hyperactivity. Some epidemiologic studies have examined a composite index of multiple behaviors, and a few studies have examined physician-diagnosed ADHD. The evidence for each of these outcomes is described below individually to the extent possible. Tests in animals can measure aspects of both attention and impulsivity; however, in several tests, a particular behavior that is observed can be identified as predominately reflecting a decrease in attention or increase in impulsivity. Therefore, the animal evidence is discussed separately according to the dominant process inferred to characterize a particular behavior measured by a neuropsychological test.

Epidemiologic Studies of Attention in Children

Among the various externalizing behaviors examined with Pb, most of the evidence for a relationship with Pb exposure is for measures of attention. Attention is the ability to maintain a consistent focus on an activity or relevant stimuli and can be assessed by examining sustained attention, concentration or distractibility. Several epidemiologic studies reviewed in the 2006 Pb AQCD (U.S. EPA, 2006b) reported associations between higher blood or tooth Pb levels with attention decrements in children from 1st grade to age 17 years, including prospective studies described in previous sections for cognitive function (Ris et al., 2004; Fergusson et al., 1993; Leviton et al., 1993). As described in this section, most recent studies also found associations of higher blood Pb levels with attention decrements in children ages 7-17 years. Many previous studies of attention included children with higher blood Pb levels than most current U.S. children but had strengths including prospective design and greater consideration for potential confounding. Some recent studies found blood Pb-associated attention decrements in populations of children with mean concurrent blood Pb levels 2 to 5 µg/dL (Cho et al., 2010; Nicolescu et al., 2010; Plusquellec et al., 2010; Chiodo et al., 2007) but in contrast with prospective studies had limitations including cross-sectional design, the potential influence of higher past Pb exposures, and consideration of fewer potential confounding factors. In the collective body of literature, most evidence was for attention rated by teachers, parents, or blinded examiners; however, associations were consistently found for more objective measures such as performance on the continuous performance test (CPT) (Figure 4-9 and Table 4-11). Thus, evidence does not indicate undue influence by biased reporting of attention decrements by parents of children with higher Pb exposures. In support of the epidemiologic findings for attention decrements in children, some

animal studies have found Pb exposure to induce less accurate signal detection in tests with distracting stimuli, which can reflect impaired attention (discussed below).

Most prospective studies that examined attention found associations with blood or tooth Pb level and had several strengths. With prenatal blood Pb, blood Pb measured 9-11 years before attention, lifetime average blood Pb, or tooth Pb, prospective studies characterized the temporal sequence between Pb exposure and attention decrements better than cross-sectional studies and made reverse causation a less likely explanation for the observed associations. These studies recruited cohorts from schools or prenatal clinics and had moderate to high follow-up participation that was not conditional on blood or tooth Pb levels, which reduces the likelihood of selection bias. Not all results were statistically significant but overall they showed a pattern of lower attention with higher blood or tooth Pb level (Figure 4-9 and Table 4-11). Most of these prospective studies were reviewed in the 2006 Pb AQCD and examined populations with mean blood Pb levels 7-14 µg/dL.



[a]Standard errors were estimated from p-values or sufficient data were not available to calculate 95% CIs.

Note: Regression coefficients were reported as T- or factor scores or were scaled to their standard deviation to facilitate comparisons among tests with different scales. Small effect estimates do not necessarily indicate lack of effect or weak effect. Results are categorized by outcome category. Where available, results from objective tests are presented first. Within categories, results generally are grouped according to strength of study design, representativeness of the study population characteristics and blood Pb levels examined, and extent of potential confounding considered. There is not necessarily a continuum of decreasing strength across studies. Effect estimates are standardized to a 1 µg/dL increase in blood Pb level in the interval from the 10th to the 90th percentile, or to 10 µg/dL, which ever is lower. The percentiles are estimated using various methods and are approximate values. Effect estimates are assumed to be linear within the interval of blood Pb level examined. Orange, blue, black, and gray symbols represent associations with prenatal maternal, childhood several years before outcome, concurrent, and lifetime average blood Pb levels, respectively.

Figure 4-9 Associations of blood Pb levels with attention, impulsivity, and hyperactivity in children.

Table 4-11 Additional characteristics and quantitative results for studies presented in Figure 4-9.

Study	Study Population and Methodological Details Organized by outcome then strength of study methodology, generalizability[a]	Blood/Tooth Pb Metrics Analyzed (µg/dL or µg/g)	Outcomes Analyzed	Effect Estimate (95% CI)[b]
Bellinger et al. (1994a)	79 young adults followed from 1st-2nd grade (1975-1978) to ages 19-20 yr, Chelsea/Somerville, MA **Prospective**. Low follow-up participation. Participation from higher SES and initial IQ and nonwhite but no effect on association with tooth Pb level. Log linear regression model adjusted for parental IQ, sex, SES, current drug, alcohol and illicit drug use, maternal education and age, birth order. Also considered potential confounding by other unspecified factors.	Deciduous tooth (1st/2nd grade) in ppm Q1: 2.9-5.9 Q2: 6.0-8.7 Q3: 8.8-19.8 Q4: 19.9-51.8	Mean (SE) Correct Responses per quartile	Q1: 98.0 (1.0)[c] Q2: 97.6 (1.1) Q3: 96.9 (1.1) Q4: 94.6 (1.1)
			Mean (SE) Reaction Time Errors per quartile Continuous Performance Test (CPT), ages 19-20 yr	Q1: 361.2 (16.5) Q2: 374.1 (17.3) Q3: 370.7 (17.9) Q4: 385.0 (17.1)
Ris et al. (2004)	195 children followed prenatally (1979-1985) to age 15-17 yr, Cincinnati, OH **Prospective**. Recruitment at prenatal clinic. High follow-up participation, no selective attrition. Mostly African-American. Linear regression model adjusted for SES, maternal IQ, HOME score, adolescent marijuana use, child sex, birth weight. Also considered potential confounding by birth outcomes, maternal age, prenatal smoking, alcohol, marijuana, and narcotics use, gravidity, # previous abortions, stillbirths, parity, caregiver education, public assistance, child age, health, and Fe status.	Mean (SD) Prenatal maternal: 8.3 (3.8) Age 3-60 mo avg: 13 (6.1) Age 78 mo: 8.3 (4.8) See Wright et al. (2008) Intervals analyzed: 4.3, 7.0, 3.6 (10th percentiles) -10	Decrement in attention factor of CPT outcomes, ages 15-17 yr Prenatal (maternal) Age 3-60 mo avg Age 78 mo	 0.16 (0.04, 0.27) 0.11 (0.03, 0.19) 0.12 (0.02, 0.22)
Cho et al. (2010)	639 children ages 8-11 yr, born 1997-2000, Seoul, Seongnam, Ulsan, Incheon, Yeoncheon, Korea **Cross-sectional**. School-based recruitment, moderate participation rate. Log linear regression model adjusted for age, sex, paternal education, maternal IQ, child IQ, birth weight, urinary cotinine, residential area. Did not consider potential confounding by parental caregiving quality.	Concurrent blood Mean (SD): 1.9 (0.67) Interval analyzed: 1.2-2.8 = 10th-90th percentiles	Commission errors, CPT Response time, CPT Total ADHD rating, teacher Total ADHD rating, parent Korean ADHD Rating Scale IV Ages 8-11 yr	0.02 (0, 0.04)[d] -0.01 (-0.03, 0.02)[d] 0.04 (0.02, 0.07)[d] 0.01 (-0.01, 0.03)[d]

Table 4-11 (Continued): Additional characteristics and quantitative results for studies presented in Figure 4-9.

Study	Study Population and Methodological Details Organized by outcome then strength of study methodology, generalizability[a]	Blood/Tooth Pb Metrics Analyzed (µg/dL or µg/g)	Outcomes Analyzed	Effect Estimate (95% CI)[b]
Nicolescu et al. (2010)	83 children ages 8-12 yr (born 1995-1999), Bucharest and Pantelimon, Romania **Cross-sectiona**l. Pantelimon near former metal processing plant. Low correlations blood Pb with blood Al, Hg. No information on participation rate. Log linear regression model adjusted for city, sex, age, computer experience, handedness, eye problems, # siblings, parental education, prenatal smoking and alcohol use, parent ever having psychological/psychiatric problem (for go/no go).	Concurrent blood Median (25th-75th): 3.7 (2.5-5.1) Interval analyzed: 2.6-8.4 = 10th-90th percentiles	Go/no go, KITAP Inattention parent rating Inattention teacher rating Hyperactivity, parent rating Hyperactivity, teacher rating German Conners Rating Ages 8-12 yr	8.2% (-0.02, 21)[e] 1.2% (-3.5, 6.7) 4.1% (-0.02, 10) 5.0% (0, 12) 4.1% (-0.02, 11)
Nigg et al. (2008)	150 children ages 8-17 yr, Birth yr and location not reported **Case-control study** of ADHD. Recruitment by mailings, outreach to clinics, community advertisements. Could have biased participation by Pb exposure leading to biased behavioral ratings. Regression-based path analysis adjusted for sex and income. Did not consider potential confounding by parental education or caregiving quality.	Concurrent blood Mean (SD): 1.03 (0.49) Detection limit=0.3 Interval analyzed: 0.5-1.7 = 10th-90th percentiles	Stop task Hyperactivity/impulsivity Teacher, parent rating, Child Behavioral Checklist, ADHD Rating Scale, Conners Rating Scale Ages 8-17 yr	0.38 (0.16, 0.60)[d] 0.21 (0, 0.42)[d]
Needleman et al. (1979)	158 children in 1st/2nd grade (1975-1978), Chelsea, Somerville, MA **Cross-sectiona**l. Recruitment from schools. Only 15% selected based on low and high tooth Pb levels. Low participation rate but no selective participation based on tooth Pb or teacher ratings. Analysis of covariance adjusted for paternal SES, parental IQ, and maternal age, # pregnancies, and education. Did not consider potential confounding by parental caregiving quality.	Tooth (1st/2nd grade) High >20 ppm (n = 58) Low <10 ppm (n = 100)	Reaction time, 12 sec delay % negative response, teacher rating Impulsive Hyperactive	Mean (SD) seconds[c] Low tooth Pb: 0.41 (0.09) High tooth Pb: 0.47 (0.12) High vs. low tooth Pb 25 vs. 9%, p = 0.01 16 vs. 6%, p = 0.08

Table 4-11 (Continued): Additional characteristics and quantitative results for studies presented in Figure 4-9.

Study	Study Population and Methodological Details Organized by outcome then strength of study methodology, generalizability[a]	Blood/Tooth Pb Metrics Analyzed (µg/dL or µg/g)	Outcomes Analyzed	Effect Estimate (95% CI)[b]
Chiodo et al. (2007)	451-466 children, age 7 yr (born 1989-1991), Detroit, MI area. **Cross-sectional**. Recruitment at prenatal clinic. 100% African-American. High prevalence prenatal drug exposure. High participation rate. Linear regression model adjusted for child sex, prenatal marijuana use (commission errors), caregiver education, HOME, maternal IQ, cocaine use, prenatal alcohol use (omission errors), child age and sex (hyperactivity), child age and sex, SES, HOME, caretaker education, maternal age, prenatal alcohol use, current marijuana use (attention problems). Also considered potential confounding by # children in home, caretaker marital status, concurrent alcohol/week and cigarettes/day, caregiver concurrent psychological symptoms, and maternal custody and prenatal cigarettes/day	Concurrent blood Mean (SD): 5.0 (3.0) Interval analyzed: 2.1-8.7 = 10th-90th percentiles	Commission Errors (%), CPT Omission Errors (%), CPT Attention problems, teacher rating, PROBS-14 Hyperactivity, Achenbach Teacher Report Form Age 7 yr	-0.08, p >0.05[f] 0.18 (0.04, 0.32)[d] 0.13 (0.03, 0.23)[d] 0.13 (0.03, 0.23)[d]
Chiodo et al. (2004)	164-236 children, age 7.5 yr, Detroit, MI area **Cross-sectional.** Recruitment at prenatal clinic. 100% African-American. High prevalence prenatal alcohol and drug exposure. Moderate participation rate. Log linear regression model adjusted for SES, # children <age 18 yr caregiver vocabulary (# errors CPT); SES, caregiver vocabulary, child age (commission errors, CPT); caregiver education, child sex, and prenatal cocaine exposure (reaction time); HOME, child sex, prenatal alcohol exposure (Inattention rating). Also considered potential confounding by family functioning, maternal prenatal marijuana or smoking exposure, parity, crowding, child life stress, caregiver age, life stress, and psychopathology, conflict tactics.	Concurrent blood Mean (SD): 5.4 (3.3) Interval analyzed: 2.2-9.5 = 10th-90th percentiles	Number of errors, visual CPT Commission errors, visual CPT Reaction time, visual CPT Inattention Factor, Barkley-DuPaul Scale Age 7.5 yr	0.35 (0, 0.69)[d] 0.05, p >0.05[f] 0.25, p >0.05[f] 0.44 (0, 0.89)[d]

Table 4-11 (Continued): Additional characteristics and quantitative results for studies presented in Figure 4-9.

Study	Study Population and Methodological Details Organized by outcome then strength of study methodology, generalizability[a]	Blood/Tooth Pb Metrics Analyzed (µg/dL or µg/g)	Outcomes Analyzed	Effect Estimate (95% CI)[b]
Fergusson et al. (1993)	878 children followed from birth to age 13 yr, Christchurch, New Zealand **Prospective.** Moderate follow-up participation, attrition did not affect results. Log linear regression model adjusted for sex, maternal education, family size, HOME score, # schools attended. Also considered potential confounding by ethnicity, SES, maternal age, breastfeeding duration, paternal education, parental smoking, child birth outcomes, residence on busy roads, weatherboard housing.	Tooth (age 6-8 yr) Mean (SD): 6.2 (3.7) µg/g	Inattention/restlessness and Conners', parent and teacher ratings Age 13 yr	0.06 (0, 0.12)[c,d]
Chandramouli et al. (2009)	488 children followed from age 4 mo (born 1992) to ages 7-8 yr, Avon, U.K. **Prospective**. All births in area eligible. Similar characteristics as U.K. census, high participation at baseline and follow-up. Participants had better educated mothers, who smoked less, better home environment. Log linear regression model adjusted for maternal education and smoking, home ownership, home facilities score, family adversity index, paternal SES, parenting attitudes at 6 mo, child sex. Also considered potential confounding by child IQ.	Age 30 mo blood Not reported		OR for increased score
		Group 1: 0-<2	Selective attention decrements	Reference
		Group 2: 2-<5	Test of Everyday Attention for Children Ages 8 yr	1.03 (0.66, 1.61)[c]
		Group 3: 5-<10		0.99 (0.62, 1.57)[c]
		Group 4: >10		1.14 (0.54,2.40)[c]
		Group 1: 0-<2	Hyperactivity, teacher Strengths and Difficulties Questionnaire, Ages 7 yr	Reference
		Group 2: 2-<5		0.84 (0.47, 1.52)[c]
		Group 3: 5-<10		1.25 (0.67, 2.33)[c]
		Group 4: >10		2.82 (1.08, 7.35)[c]
Leviton et al. (1993)	1,923 children followed from birth (1979-1980) to age 8 yr, Boston, MA area **Prospective**. Recruited from birth hospital. Moderate participation at baseline and follow-up. Regression model adjusted for single parent family, gestational age, # children in home, maternal education, ethnicity, daycare in 1st 3 years. Also considered potential confounding by other unspecified factors	Prenatal (cord) blood Mean: 6.8 Tooth Pb (Age 6 yr) Mean: 3.3 µg/g	Daydreaming problems, Boston Teacher Questionnaire, age 8 yr	RR (yes/no) per natural log increase
			Prenatal (cord)	Girls: 1.3 (0.8, 2.2)[c]
				Boys: 1.0 (0.6, 1.5)[c]
			Tooth Pb	Girls: 1.5 (0.9, 2.6)[c]
				Boys: 1.1 (0.7, 1.7)[c]

Table 4-11 (Continued): Additional characteristics and quantitative results for studies presented in Figure 4-9.

Study	Study Population and Methodological Details Organized by outcome then strength of study methodology, generalizability[a]	Blood/Tooth Pb Metrics Analyzed (µg/dL or µg/g)	Outcomes Analyzed	Effect Estimate (95% CI)[b]
Burns et al. (1999)	322 children followed from birth (1979-1982) to ages 11-13 yr, Port Pirie, Australia. **Prospective**. Moderate follow-up participation. Participants had higher birth weight, older mothers, less educated fathers. Log linear regression model adjusted for maternal age, prenatal smoking status, IQ, concurrent psychopathology, and education, birth weight, type of feeding, length of breastfeeding, paternal education and occupation, birth order, family functioning, parental smoking, marital status, HOME score, child IQ.	Lifetime avg (to age 11-13 yr) blood GM (5th-95th) Boys: 14.3 (13.5-15.1) 10th-90th: 13.7-14.9 Girls: 13.9 (13.2-14.6) 10th-90th: 13.4-14.5	Attention problems, boys Attention problems, girls Maternal rating by Child Behavior Checklist Ages 11-13 yr	0.02 (-0.04, 0.08) 0.07 (0.02, 0.12)
Wasserman et al. (2001)	191 children followed prenatally (1984-1985) to age 5 yr, Pristina, Yugoslavia. **Prospective**. High follow-up participation. Participants had lower maternal education, were Albanian, had higher average blood Pb at age 4 yr. Generalized estimating equations with log blood Pb adjusted for child sex, birth weight, and age, ethnicity, HOME score, maternal education and smoking. Did not consider potential confounding by maternal IQ.	Lifetime avg (to age 5 yr) blood Mean (SD): 7.2 Only log blood Pb reported	Attention problems Maternal rating, Child Behavior Checklist Repeated measures ages 4-5 yr.	Change in raw score per log increase in blood Pb: 0.06 (-0.21, 0.33)[c]
Chen et al. (2007)	721 children in TLC trial followed from ages 1-3 to 5 yr, Baltimore, MD; Cincinnati, OH; Newark, NJ; Philadelphia, PA **Prospective**. Mostly African-American. Multi-city, high participation rate. Eligible for chelation trial because of high age 1-3 yr blood Pb levels (20-44 µg/dL). Linear regression-based path analysis adjusted for city, race, sex, language, single parent, parental education and employment, age at blood Pb measurement, caregiver IQ. Considered potential confounding by chelation treatment but not parental caregiving quality. Direct = independent of IQ. Indirect = mediated through IQ.	Age 2 year blood Mean (SD): 26 (5.1) Interval analyzed: 20-33 =10th-90th percentiles	Hyperactivity Index ADHD index	Direct: 0.07 (-0.07, 0.20) Indirect: 0.03 (0, 0.05) Direct: 0.04 (-0.09, 0.18) Indirect: 0.05 (0.03, 0.13)
		Concurrent blood Mean (SD): 12 (5.2) Interval analyzed: 6.6 (10th percentile)-10	Hyperactivity Index ADHD index Parent ratings, Conners Scale-Revised, Age 5 yr	Direct: 0.08 (-0.06, 0.21) Indirect: 0.04 (-0.06, 0.13) Direct: 0.04 (-0.10, 0.18) Indirect: 0.07 (0.03, 0.11)

Table 4-11 (Continued): Additional characteristics and quantitative results for studies presented in Figure 4-9.

Study	Study Population and Methodological Details Organized by outcome then strength of study methodology, generalizability[a]	Blood/Tooth Pb Metrics Analyzed (µg/dL or µg/g)	Outcomes Analyzed	Effect Estimate (95% CI)[b]
Canfield et al. (2003b)	96-127children born 1994-1995 followed ages 6 mo-4-4.5 yr, Rochester, NY **Cross-sectiona**l. Recruitment from study of dust control. Mostly nonwhite. Moderate follow-up participation, no comparison of nonparticipants. Linear mixed effects model adjusted for child age, gestational age, maternal IQ and education, HOME, race, color/shape knowledge, child IQ (both outcomes). Plus: race (control phase), child sex (inhibit phase). Also considered potential confounding by birth weight, household income, prenatal smoking exposure.	Age 4 yr blood Mean: 6.5 Range: 1.7-21 10th-90th: data not available	Attention decrement, control phase Attention decrement, inhibit phase Examiner rating during Shape School Task Repeated measures at ages 4 and 4.5 yr	0.01 (-0.01, 0.04) 0.008 (-0.02, 0.04)
Silva et al. (1988)	535 children age 11 yr (born 1972-1973), Dunedin, New Zealand **Cross-sectional.** Moderate participation rate. Participants were of higher SES and non-Maori. Log linear regression adjusted for SES, maternal verbal skills, age at 1st birth, and mental health symptoms, change in residence and school, solo parenting, child/parent separation, family relations, marriage guidance sought, child sex, birth order, and IQ. Did not consider potential confounding by parental caregiving quality.	Concurrent blood Mean (SD): 11 (4.9) Interval analyzed: 5.9 (10th percentile)-10	Inattention, parent Inattention, teacher Hyperactivity, parent Hyperactivity, teacher Rutter Behavior Questionnaire Age 11 yr	0.06 (-0.03, 0.16) 0.15 (0.06, 0.25) 0.13 (0.03, 0.23) 0.12 (0.01, 0.22)
Plusquellec et al. (2010)	84-98 children, ages 5-6 years (born 1994-1997), Inuit communities, Quebec, Canada **Cross-sectional.** Study of multiple exposures. Low but no selective participation by blood Pb, PCBs, or Hg. Log linear regression model adjusted for birth weight, sex, parity, caregiver education (impulsivity); birth weight, SES, child blood Hg (off task duration), maternal prenatal binge drinking, # children in home,cord and child serum fatty acids (global activity rate). Also examined potential confounding by,# residents per room, caretaker psychological distress, nonverbal reasoning, and linguistic acculturation, HOME, maternal prenatal illicit drug use and cigarettes/day, serum Se. p > 0.20 for associations with blood Hg or PCBs.	Concurrent blood Mean (SD): 5.4 (5.0) Interval analyzed: 1.4-11 = 10th-90th percentiles	Off task duration Impulsivity Global activity rate Examiner ratings, modified Infant Behavior Rating Scale Ages 5-6 yr	0.02 (0, 0.04)[d] 0.02 (0, 0.04)[d] 0.014 (-0.01, 0.03)[d]

Table 4-11 (Continued): Additional characteristics and quantitative results for studies presented in Figure 4-9.

Study	Study Population and Methodological Details Organized by outcome then strength of study methodology, generalizability[a]	Blood/Tooth Pb Metrics Analyzed (µg/dL or µg/g)	Outcomes Analyzed	Effect Estimate (95% CI)[b]
Kordas et al. (2007)	157 children ages 6-8 yr (born 1993-1995), Torreon, Mexico **Cross-sectional.** 26% selected from larger study for classroom observation. Residence near metal foundry. Linear regression model adjusted for age, sex, SES, home ownership, crowding in home, maternal education, child living with 1 or both parents, forgetting homework. Also considered potential confounding by micronutrients but not parental caregiving quality.	Concurrent blood Mean (SD): 12 (6.1) Interval analyzed: 5.4 (10th percentile)-10	Off task passive behavior Examiner rating, instrument developed by investigator Ages 6-8 yr	0.034 (0.01, 0.06)
Roy et al. (2009)	756 children ages 3-7 yr (born 1998-2003), Chennai, India **Cross-sectional**. Recruitment at schools. No information provided on participation. Log linear regression model adjusted for age, sex, average monthly income, hemoglobin, parental education, # other children, cluster of school and classroom. Did not consider potential confounding by parental caregiving quality.	Concurrent blood Mean (SD): 11 (5.3) Interval analyzed: 5.8 (10th percentile)-10	Inattentive z-score Hyperactive z-score ADHD index z-score Teacher ratings, Conners' ADHD/DSM-IV Scales Ages 3-7 yr	0.03 (0.01, 0.06) 0.02 (-0.01, 0.04) 0.02 (0, 0.05)
Rabinowitz et al. (1992)	493 children, grades 1-3, Taiwan **Cross-sectional.** Some reside near smelter. Recruitment from schools. High participation rate. Logistic regression model adjusted for sex, # adults at home. Also considered potential confounding by grade, child longest hospital stay parental education, SES, birth outcomes, handedness, language at home, prenatal maternal medicine, alcohol, and smoking but not parental caregiving quality.	Tooth (grades 1-3) Mean (SD): 4.6 (3.5)	Hyperactivity Syndrome Boston Teacher Questionnaire Grades 1-3	OR vs. <2.3 µg/g as reference 2.3-7 µg/g: 1.9 (0.53, 6.5)[c] >7 µg/g: 2.8 (0.68, 12)[c]

Table 4-11 (Continued): Additional characteristics and quantitative results for studies presented in Figure 4-9.

Study	Study Population and Methodological Details Organized by outcome then strength of study methodology, generalizability[a]	Blood/Tooth Pb Metrics Analyzed (µg/dL or µg/g)	Outcomes Analyzed	Effect Estimate (95% CI)[b]
Froehlich et al. (2009)	2,588 children, ages 8-15 yr (born 1986-1996), U.S. NHANES 2001-2004 **Cross-sectional**. U.S. representative results, study of multiple risk factors and outcomes. Logistic regression adjusted for child serum cotinine, sex, age, preschool attendance, birth weight, income/poverty ratio, maternal age and prenatal smoking, race/ethnicity. Did not consider potential confounding by parental education or caregiving quality.	Concurrent blood Tertile 1: 0.2-0.8 Tertile 2: 0.9-1.3 Tertile 3: >1.3 1% below detection limit of 0.3	ADHD DSM-IV criteria met (yes/no) Parental rating, ADHD module of Diagnostic Interview Schedule for Children Age 8-15 yr	OR vs. 0.2-0.8 µg/dL as reference 0.9-1.3 µg/dL: 1.7 (0.97, 2.9)[g] >1.3 µg/dL: 2.3 (1.5, 3.8)[g]

[a]Results are organized by outcome examined, then grouped by strength of study design, representativeness of the study population characteristics and blood Pb levels examined, and extent of potential confounding considered. There is not necessarily a continuum of decreasing strength across studies.

[b]Effect estimates are standardized to a 1 µg/dL increase in blood Pb level in the interval from the 10th percentile of blood Pb level to 10 µg/dL or the 90th percentile, whichever is lower and scaled to the standard deviation of the test score to facilitate comparisons among tests that are scored on different scales. For studies with 10th percentiles of blood Pb level > 10 µg/dL, effect estimates are standardized to a 1 µg/dL increase in blood Pb level in the interval from the 10th to 90th percentile of blood Pb level.

[c]Results not presented in Figure 4-9 because tooth Pb analyzed or data reported only for log blood Pb.

[d]Standard error was estimated from the reported p-value.

[e]Results represent the change in false alarm rate.

[f]Sufficient data were not available to calculate 95% CIs.

[g]Results not presented in Figure 4-9 because OR or RR reported in papers.

Prospective studies in Cincinnati and in Chelsea/Somerville, MA found Pb-associated attention decrements in adolescents and young adults as indicated by increases in commission and omission errors or reaction time on the objective CPT (Ris et al., 2004; Bellinger et al., 1994a). In the CPT, subjects are assessed for their ability to maintain focus during a repetitive task and respond to targets or inhibit responses. Stronger, more consistent results were found in the larger Cincinnati cohort with the analysis of blood Pb levels as a continuous variable. In 195 mostly African-American, lower SES adolescents ages 15-17 years, Ris et al. (2004) found attention decrements (composite of CPT outcomes) in association with higher prenatal maternal, age 3-60 month average, and age 78 month blood Pb levels, particularly among males. The mean (SD) blood Pb levels as reported in Wright et al. (2008) were 8.3 (3.8) µg/dL for maternal prenatal, 13.4 (6.1) for age 3-60 month average, and 8.3 (4.8) for age 78 month. Among 79 primarily white, higher SES young adults ages 19-20 years, Bellinger et al. (1994a) found that compared with the group with 1st/2nd grade tooth Pb levels 2.9-5.9 ppm, the group with tooth Pb levels >19.9 ppm had fewer correct responses on the CPT and had longer reaction times for correct responses but did not commit more commission errors (responding to a nontarget) ($p = 0.25$ for overall sustain factor). Although blood Pb levels at older ages were not examined and results do not exclude an effect of more recent Pb exposures, the combined evidence from these prospective CPT studies of points to an effect on attention of Pb exposures from earlier in development.

In addition to objective tests of attention, most prospective studies found Pb-associated attention decrements in school-aged children as rated by teachers, parents, or independent examiners (Figure 4-9 and Table 4-11). In children ages 7-13 years in Australia, New Zealand, and Boston, MA, lower ratings of attention were associated with higher lifetime (to age 11-13 years) average (Burns et al., 1999), prenatal cord (Leviton et al., 1993), and tooth (collected at ages 6-8 years) Pb levels (Fergusson et al., 1993; Leviton et al., 1993). The mean blood Pb levels in these populations were 13.9 µg/dL for lifetime average (Burns et al., 1999) and 6.8 µg/dL for prenatal cord (Leviton et al., 1993). In separate cohorts, associations of lifetime average blood Pb level (Burns et al., 1999) and tooth Pb level (Leviton et al., 1993) with attention ratings were stronger among girls than boys. Other prospective studies found lack of association with early childhood or lifetime average blood Pb levels. Age 30 month blood Pb levels was not associated with teacher rating of selective inattention in U.K. children ages 7-8 years (Chandramouli et al., 2009). Lifetime average blood Pb level was not associated with maternal rating of attention problems in the Yugoslavia cohort at age 4-5 years (Wasserman et al., 2001). However, ratings may be less reliable in younger children, in whom patterns of behavior are less well established.

Another strength of prospective studies, both those finding and not finding associations, was their examination of many potential confounding factors, either by inclusion in statistical models with Pb or by examination in preliminary analyses. Higher blood or tooth Pb levels were associated with attention decrements with adjustment for parental education, IQ, and caregiving quality as well as SES in most studies (Ris et al., 2004; Burns et al., 1999; Fergusson et al., 1993) and in fewer studies, birth outcomes and exposures to smoking, alcohol, or drugs. These results increase confidence that the associations observed with Pb biomarkers reflect a relationship with Pb exposure.

Most cross-sectional studies found associations between higher concurrent blood, tooth, or hair Pb level and attention decrements among children ages 3-16 years. With analysis limited to concurrently measured Pb biomarkers, the directionality of effects is difficult to characterize. While these studies considered potential confounding by SES, child age, sex, family structure, or parental education, most did not examine potential confounding by parental caregiving quality. Cross-sectional studies comprised a mix of previously reviewed and recently published studies and a range of concurrent blood Pb levels, means 1-13.2 µg/dL (Table 4-11). Among studies of children with mean concurrent blood Pb levels 11.1-13.2 µg/dL, most (Roy et al., 2009; Kordas et al., 2007; Silva et al., 1988) but not all (Liu et al., 2011b) found associations with lower attention ratings. Associations with blood or hair Pb levels were found in most of the study populations that lived near metal sources in Mexico or China (Liu et al., 2011b; Bao et al., 2009; Kordas et al., 2007). Needleman et al. (1979) found longer reaction time at varying intervals of delay and higher ratings of distractibility in children in grades 1-2 in Chelsea and Somerville, MA with tooth Pb levels > 24 ppm than those with tooth Pb <6 ppm (Table 4-11). Some studies included young children (e.g., ages 3, 4 years), in whom behavioral ratings may be less reliable (Liu et al., 2011b; Roy et al., 2009).

Evidence of Pb-associated attention decrements in populations with mean concurrent blood Pb levels 1-6.5 µg/dL (ages 4-17 years) was more variable. A large group of these studies considered several potential confounding factors including SES, parental education, parental IQ, and parental caregiving quality (Plusquellec et al., 2010; Chiodo et al., 2007; 2004; Canfield et al., 2003b). These studies also had population-based recruitment and moderate to high follow-up, without indication of biased participation by children with higher blood Pb levels and poorer attention. However, in these higher-quality studies of children with lower blood Pb levels, there was a difference in consistency of evidence between studies of younger children ages 4-5 years and older children ages 7 years. Chiodo et al. (2007; 2004) examined children ages 7 years in Detroit, MI and found concurrent blood Pb-associated poorer performance on several tests of attention on the CPT and lower teacher or study examiner ratings of attention. In these populations with high prevalence of prenatal alcohol or drug exposure, results did

not indicate confounding by these other exposures. However, the presence of these other exposures may limit the generalizability of results.

Among the lower concurrent blood Pb level studies that included younger children, ages 4-5 years, evidence was mixed. An association between higher concurrent blood Pb level and attention decrements was found among Inuit children ages 5-6 years in Quebec, Canada (Plusquellec et al., 2010) but not the Rochester cohort (Canfield et al., 2003b). These results were based on maternal or study examiner ratings. Ratings may be less reliable in younger children, in whom patterns of behavior are less well established. Canfield et al. (2003b) initially found associations between higher concurrent blood Pb level and lower ratings of attention, but they were attenuated with adjustment for child color and shape knowledge and age 3-year FSIQ, which suggested that poorer knowledge of the task parameters may increase distraction. However, Cho et al. (2010) found an association between concurrent blood Pb level and lower attention ratings with adjustment for child IQ, indicating the relationship between attention and cognitive function may vary across populations. In the study of Inuit children, concurrent blood Pb level but not cord blood Pb level was associated with duration of off-task behavior as rated by study examiners (Plusquellec et al., 2010). In this population with potential co-exposures to polychlorinated biphenyls (PCBs) and mercury (Hg) from high consumption of fish, blood levels of PCBs and Hg were reported not to be associated with off task duration, reducing the likelihood of confounding by these co-exposures.

Other studies examining lower concurrent blood Pb levels, means 1.9 and 3.7 µg/dL, found associations with attention decrements in children ages 8-12 years as assessed by CPT or parent/teacher ratings (Cho et al., 2010; Nicolescu et al., 2010). Nigg et al. (2008) did not provide quantitative results but only reported that the association between concurrent blood Pb level (mean 1 µg/dL, detection limit=0.3 µg/dL) and inattention-disorganization in children ages 8-17 years lost statistical significance with adjustment for sex and family income. Despite some evidence for attention decrements at relatively low blood Pb levels, these studies had several limitations in addition to their cross-sectional design. In these older children, higher earlier childhood Pb exposures may have contributed to observed associations. These studies adjusted for parental education but not consistently for other SES factors. They did not consider potential confounding by parental caregiving quality. Further, sufficient information was not reported to assess whether participation was biased to those with higher Pb exposure and attention decrements. Among children ages 8-11 years in Korea, higher concurrent blood Pb level (mean 1.9 µg/dL, detection limit=0.58 µg/dL) was associated with a higher teacher rating of inattention and more commission errors on the CPT but not other parameters of the CPT (Figure 4-9 and Table 4-11) (Cho et al., 2010). Urinary cotinine was more strongly associated with CPT performance than blood Pb level and the primary cause of

attenuation of blood Pb effect estimates. Past Pb exposures especially may have had an influence in the study of children ages 8-12 years in Romania, 55% (n = 46/83) of whom lived near a former metal processing plant and had higher concurrent blood Pb levels (mean 5.1 versus 3.2 µg/dL) (Nicolescu et al., 2010). Among all subjects, a 1 µg/dL increase in concurrent blood Pb level in the 10th to 90th percentile interval (1.8-7.1 µg/dL) was associated with a 5.2% higher (95% CI: -0.02, 13%) teacher rating of inattention and a weaker, imprecise 1.5% (95% CI: -4.4, 8.5) higher parent rating. Blood Pb levels were poorly correlated (r = -0.06, 0.05) with blood levels of aluminum (Al) or Hg, and neither of these other metals was associated with attention ratings. Results did not change substantially in an analysis that excluded the 5 children with blood Pb levels ≥ 10 µg/dL.

Toxicological Studies of Attention

In support of the associations described in the preceding sections for blood Pb level with attention decrements in children, studies have found Pb-induced decreases in attention in animals, although results have not been consistent across studies. Although tests in animals can measure aspects of both attention and impulsivity, behaviors measured with signal detection tests with distraction can be inferred as predominately assessing sustained attention. In this test, animals earn food rewards by responding to a target stimulus and not responding to a distracter light. Poorer sustained attention and greater distractibility are indicated by lack of response to the target and increased response to the distracter light, respectively. The 2006 Pb AQCD (U.S. EPA, 2006b) reported inconsistent effects of Pb exposure in animals on performance in this test. For example, postweaning Pb exposure that produced blood Pb levels of 16 and 28 µg/dL induced small decreases in attention in adult rats as indicated by small increases in omission and commission errors but only during sessions with long intervals between stimuli (Brockel and Cory-Slechta, 1999a). Lifetime Pb exposure from birth (blood Pb levels 15, 25 µg/dL) was found to induce distractibility in monkeys at age 9-10 years as indicated by increased responding to irrelevant cues, i.e., distracting stimuli, in a spatial discrimination reversal task. Repeated reversal testing revealed that these deficits likely were not due to sensory or motor impairment (Gilbert and Rice, 1987).

In animals, Pb-induced decrements in attention have been inferred from tests that were designed to assess attention but that have elicited behaviors that suggest deficits in attention. For example, a study reported that impaired performance on auditory threshold tasks in Pb-exposed monkeys was likely due to lack of attention (Laughlin et al., 2009). Rhesus monkeys were exposed to Pb acetate from gestation (drinking water of mothers, 3 months prior to mating) to birth or postnatally from birth to age 5.5 months at weaning and had resultant bone Pb levels at 11 years of 7 and 13 µg/g for prenatal and postnatal

groups, respectively, and average blood Pb levels during Pb exposure of 35 and 46 µg/dL, respectively. Animals were tested at age 13 years when blood Pb levels had returned to baseline levels. The inability of some of the monkeys to engage or focus attention on the task at hand yielded fewer available measurements in Pb-exposed animals versus controls. These observations were made in monkeys with higher peak blood Pb levels than those relevant to this ISA.

Epidemiologic Studies of Impulsivity in Children

Compared to attention, measures specific to impulsivity have been examined in few epidemiologic studies of children and mostly in recent cross-sectional studies. However, evidence in children indicates Pb-associated poorer performance on tests of response inhibition which finds coherence with similar evidence in animals. Response inhibition is a measure of impulsivity and in children has been assessed with stop signal tasks, which measures the execution of action in response to stimuli and the inhibition of that action when given a stop signal. Recent cross-sectional studies found that children with higher concurrent blood Pb levels had greater responses with stop signals. Among children ages 8-12 years in Romania, a 1 µg/dL increase in concurrent blood Pb level in the 10th-90th percentile interval 1.8-7.1 µg/dL was associated with a 10% (95% CI: 0, 16) increased false-alarm rate in responses to stop signals with adjustment for city, sex, age, computer experience, handedness, eye problems, number of siblings, parental education, prenatal alcohol and smoking exposure, and parental self report of ever having a psychopathology (Nicolescu et al., 2010). Children in one town lived near a metal processing plant; however, associations were not found with blood Pb levels of Al and Hg. In a case-control study of children with ADHD, Nigg et al. (2008) found that higher concurrent blood Pb level was associated with poorer response inhibition on a stop task

A few studies found associations between higher concurrent tooth (Needleman et al., 1979) or blood (Plusquellec et al., 2010; Nigg et al., 2008) Pb level with higher ratings of impulsivity. Nigg et al. (2008) found associations of concurrent blood Pb levels with parent and teacher ratings of a composite hyperactivity/impulsivity index in a group of children (location not reported) with and without ADHD (ages 8-17 years) with a mean blood Pb level of ~1 µg/dL (detection limit = 0.3 µg/dL). Regression-based path analysis showed that the association between blood Pb level and hyperactivity-impulsivity ratings was mediated by (i.e., occurred through) poorer performance on the more objective stop task. Path analysis produces partial regression coefficients that measure the effect of one variable on another controlling for previous variables. Because hyperactivity and impulsivity are characteristics of ADHD, ratings likely are higher among ADHD cases than controls. If the differential reporting of hyperactivity/impulsivity by ADHD status is unrelated to Pb exposure or blood Pb level, it is less likely to bias associations with Pb.

However, differential rating between cases and controls could produce an artifactual association with blood Pb level if participation by children with ADHD is biased to those with higher Pb exposures and blood Pb levels. Nigg et al. (2008) also used path analysis to show that the Pb-associated increase in hyperactivity/impulsivity was independent of an association with IQ. With adjustment for sex and income, concurrent blood Pb level was directly associated with hyperactivity/impulsivity, and the association was not completely mediated by the blood Pb-IQ association. Instead, the association between blood Pb level and IQ was found to be mediated by the association of Pb with hyperactivity/impulsivity. In a population of Inuit children at ages 5-6 years, the relationship between concurrent blood Pb level and motor function was found to be mediated by (i.e., occur through) the association of blood Pb level with impulsivity (Fraser et al., 2006).

These studies of impulsivity were similar in their adjustment for potential confounding by SES, sex, and parental education (Table 4-11). A few adjusted for smoking exposure. However, parental IQ or caregiving quality were not examined in most studies. Plusquellec et al. (2010) has the most extensive consideration for potential confounding, excluding parental IQ, HOME score, micronutrient levels, Hg, and PCBs (potential co-exposures from high consumption of fish) from final models with blood Pb based on their lack of strong association with impulsivity. There also was no biased participation according to the exposures examined. A limitation of this study is the young age of some children at examination (study range: age 5-6 years). The variable nature of behaviors in younger children may limit the implications of findings.

Toxicological Studies of Impulsivity

The associations described between higher blood Pb level and greater impulsivity in children are supported by findings in animals for Pb-induced increased perseveration and impaired ability to inhibit inappropriate responses. In animals, these effects are supported by studies reviewed in the 1986 and 2006 Pb AQCDs (U.S. EPA, 2006b, 1986b) and recent studies. Animal studies provide more consistent evidence for the effects of Pb exposure on impulsivity in animals than on sustained attention. As mentioned earlier, behaviors displayed by animals in a variety of tests can be identified as predominately reflecting impulsivity. These include tests of Differential Reinforcement of Low Rates of Responding (DRL), FI schedule performance, FI with Extinction, or Fixed Ratio (FR)/waiting-for-reward. Greater impulsivity is indicated by premature responses, decreased pause time between two scheduled events, and increased perseveration.

Behaviors observed in tests of operant conditioning with FI reinforcement schedules also have been used to indicate impaired learning in animals (Section 4.3.2.3), and the

interactions observed between Pb exposure and maternal or offspring stress also may apply to effects on impulsivity. Maternal exposure to 150 ppm Pb with and without stress co-exposure was found to increase overall FI rate and decrease PRP in rats (Table 4-7). Lifetime (from gestation) Pb exposure resulting in blood Pb levels 11-16 µg/dL increased overall FI rate without stress co-exposure and decreased PRP with stress co-exposure (Table 4-7) (Rossi-George et al., 2011). Discrimination reversal learning has been shown to be affected by Pb exposure. In these tasks, an animal is trained to choose between two alternative responses and then is required to reverse that association. Perseveration or lack of inhibition of the original response can be interpreted to involve impulsivity. Spatial and non-spatial discrimination reversal was significantly affected in monkeys after Pb exposure during infancy, after infancy, or continuously from birth, and was exacerbated with distracting stimuli (Rice, 1990; Rice and Gilbert, 1990b; Gilbert and Rice, 1987). These monkeys had blood Pb levels in the range of 15-36 µg/dL, which includes those relevant to this ISA. Contrary to the findings in monkeys, Hilson and Strupp (1997), found Pb exposure (Pb acetate in drinking water GD1-PND28, blood Pb levels 26, 51 µg/dL) in rats to slow reversal learning in an olfactory discrimination task. But, analysis of the response patterns showed that Pb exposure shortened the perseverative responding phase of reversal learning and lengthened the post-perseverative phase of chance responding, indicating impairments in associative ability not response inhibition.

The effects of Pb exposure on impulsivity also have been demonstrated as shorter time Pb-exposed animals will wait for reward in FR/waiting for reward testing. In this test, animals can obtain food by pressing a lever a fixed number of times (FR component). Then, free food is delivered at increasingly long time intervals, so long as the animal inhibits additional lever presses. Animals can reset the schedule to return to the FR component at any time. Brockel and Cory-Slechta (1998) exposed male Long-Evans rats to 0, 50, or 150 ppm Pb acetate in drinking water from weaning, which produced respective blood Pb levels of <5, 11, and 29 µg/dL after 3 months of exposure. After 40 days of exposure, the 150 ppm Pb-exposed rats responded more quickly in the FR component and reset the schedule (thus shortening the waiting period) more often than did the 50 ppm Pb-exposed rats and controls. In the waiting component, average wait time was significantly lower in both Pb exposure groups compared to controls. The rats exposed to 150 ppm Pb also had higher response rates, earned more reinforcers per session, but had a higher response-to reinforcement-ratio than did the 50 ppm Pb group and controls, which indicated less efficient responding. Mechanistic understanding of the Pb-induced effects on responding in the FR/waiting for reward test was provided by a study with similar postweaning exposure to 0, 50, and 150 ppm Pb that yielded respective blood Pb levels of <5, 10, and 26 µg/dL after 3 and 7 months of exposure. Administration of a dopamine (D2) receptor agonist reversed the Pb-induced effects on increasing FR

response rates and decreasing mean waiting time, suggesting a role for dopamine-like receptors in Pb-induced effects on waiting behavior (Brockel and Cory-Slechta, 1999b).

Pb-induced impulsivity appears to be related to emotionality, which was found in rats trained to perform an olfactory discrimination task, albeit at higher Pb exposures than those relevant to this ISA. Rats were given early postnatal Pb exposure (300 ppm Pb acetate via dam drinking water PND1-PND17 then either 20 or 300 ppm in their own drinking water PND18-PND30), which produced blood Pb levels of 40-60 and 100-140 µg/dL. The rats were tested as young adults on a food-motivated olfactory discrimination task in which rewards for correct responses were occasionally and unpredictably omitted. Pb-exposed animals were more sensitive both to their own errors and to reward omission than controls, suggesting a lowered capacity for regulating arousal and emotion. Administration of the chelator succimer after the Pb exposure period (PND31-PND52) normalized reactivity to reward omission and errors in the Pb-treated rats, but increased reactivity in the control animals (Beaudin et al., 2007).

The same laboratory using the same Pb exposure protocol also found heightened reactivity to errors in tests of visual discrimination and visual sustained attention (Stangle et al., 2007). Pb-induced increases in impulsivity were found in rats at PND62 after exposure to 300 ppm Pb (blood Pb level 31 µg/dL at PND52) but not 20 ppm Pb (blood Pb level 13 µg/dL). Impulsivity was inferred from a higher number of premature responses after the basic rules of the task had been learned. Succimer reduced blood Pb level to 3-9 µg/dL at PND52 but did not reduce premature responding at PND62, indicating persistence of the effect. Finally, monkeys exposed to Pb from birth to time of testing at age 3-4 years with blood Pb levels 15 and 25 µg/dL showed slower acquisition of a DRL schedule, which requires an animal to respond at a low rate for rewards. However, the effect was reversible. Pb-exposed monkeys eventually acquired reinforcement rates equal to those of controls (Rice and Gilbert, 1985).

In summary, several studies in animals indicate that Pb exposure of rodents and non-human primates from birth or after weaning changes behavior in ways consistent with increased impulsivity, primarily as indicated by impaired response inhibition. Some observations of Pb-induced impulsivity in animals were made with blood Pb levels considered relevant for this ISA. The observations for Pb-induced increases in impulsivity in animals provide support for associations found in children of higher blood and tooth Pb levels with lower response inhibition and higher ratings of impulsivity.

Hyperactivity

Studies reviewed in the 2006 Pb AQCD (U.S. EPA, 2006b) indicated associations between higher concurrent blood Pb level or tooth Pb level and higher parent or teacher ratings of hyperactivity in children ages 6-11 years in the U.S., Asia, and New Zealand (Rabinowitz et al., 1992; Silva et al., 1988; Gittleman and Eskenazi, 1983; Needleman et al., 1979; David et al., 1976). The case-control or cross-sectional design of studies limited understanding of the temporal sequence between Pb exposure and hyperactivity. Several recent studies, including a prospective study (Chandramouli et al., 2009), also found associations between blood Pb level and hyperactivity as rated by teachers and parents (Figure 4-9 and Table 4-11). Overall, studies indicated associations in children ages 3-12 years with mean concurrent blood Pb levels 3.7-12 µg/dL.

The recent prospective study of children in the U.K. helps address some of the limitations of cross-sectional studies by demonstrating an association between higher early childhood (age 30 months) blood Pb level and higher teacher ratings of hyperactivity later in childhood at age 7-8 years and by adjusting for several potential confounding factors, including SES, home facilities score, and family adversity index (Chandramouli et al., 2009). In addition to the prospective design, the study had high participation rates at baseline and follow-up from a population with similar characteristics as reported in the U.K. census. Increases in hyperactivity were found primarily in the group of children with blood Pb levels >10 µg/dL and were independent of associations with IQ.

The cross-sectional evidence is provided by previous and recent studies conducted in populations with a range of blood Pb levels and with variation in the extent of consideration for potential confounding. A few studies found concurrent blood Pb-associated higher ratings of hyperactivity in populations with mean concurrent blood Pb levels 5-6 µg/dL and examined potential confounding by a large number of factors. Among children ages 5-6 years in Quebec, Canada, Plusquellec et al. (2010) found that hyperactivity (assessed as observer rating and computer program) was related to concurrent blood Pb level but not HOME score, caretaker IQ or education, or other prevalent exposures in the community including Hg and PCBs. Among children age 7 years in Detroit, MI, hyperactivity was related to concurrent blood Pb levels but not SES, parental education or IQ, or HOME score (Chiodo et al., 2007). While there is confidence in the internal validity of results from these studies, the applicability to other populations may be limited. The former study included younger children in whom behavioral patterns are less fixed, and the latter study examined a population with high prevalence of prenatal drug exposure.

Other cross-sectional studies found Pb-associated increases in hyperactivity but examined fewer potential confounding factors. Adjustment for SES, maternal education and IQ was

common; however, few adjusted for parental caregiving quality. Associations were found with tooth Pb in children in grades 1-3 (Rabinowitz et al., 1992; Needleman et al., 1979). Several (Roy et al., 2009; Chen et al., 2007; Silva et al., 1988) but not all (Liu et al., 2011b) studies found associations in populations of children ages 3-11 years in the U.S., New Zealand, and Asia with relatively high concurrent blood Pb levels (means 10-12 µg/dL). Nicolescu et al. (2010) found an association in children ages 8-12 years in Romania with relatively lower concurrent blood Pb levels, median 3.7 µg/dL. Results were adjusted for several potential confounding factors including age, sex, parental education, and prenatal smoking or alcohol exposure. Hyperactivity was not related to Al or Hg, other prevalent exposures in the community. However, potential confounding by other SES variables or parental caregiving quality was not examined.

A recent study did not clearly indicate an effect of Pb on inducing hyperactivity in mice. Pb exposure during gestation and the early postnatal period (PND10) (27 and 109 ppm Pb acetate: ≤ 10 and 42 µg/dL peak blood Pb levels, respectively) increased motor activity of male mice at age 1 year with co-treatment with amphetamines but not female mice (Leasure et al., 2008). Without amphetamines, Pb induced less activity of mice, and the lower Pb dose inhibited activity more than the higher Pb dose did.

Ratings of Attention Deficit Hyperactivity Disorder-related Behaviors

In addition to finding associations with attention, impulsivity, and hyperactivity, some of the recent epidemiologic studies described in the preceding sections found associations between higher concurrent blood Pb level and higher parental and teacher ratings of ADHD-related behaviors (Cho et al., 2010; Nicolescu et al., 2010; Froehlich et al., 2009; Roy et al., 2009), calculated as a composite of the various behaviors that are evaluated in the diagnosis of ADHD. The strengths and limitations of these studies have been described in the preceding sections. Main limitations were the cross-sectional design, lack of validation of ADHD ratings with a clinical diagnosis, and lack of consideration for some potentially important confounding factors. Studies considered age, sex, SES or parental education but usually not both, and none considered parental caregiving quality. Thus, the evidence specifically for these total ADHD index ratings were emphasized less than evidence for individual behaviors in drawing conclusions about the effects of Pb exposure on attention, impulsivity, and hyperactivity.

The large, U.S. representative analysis of children participating in NHANES 2001-2004 found an association between concurrent blood Pb level in children ages 8-15 years and parental assessment of child ADHD-related behaviors using the Diagnostic Interview Schedule for Children which uses DSM-IV criteria to identify children at increased risk of meeting diagnostic criteria for ADHD (Froehlich et al., 2009). Compared with children

with concurrent blood Pb levels <0.8 µg/dL (4% below detection limit of 0.3 µg/dL), children with concurrent blood Pb levels >1.3 µg/dL had an elevated odds of parentally-rated ADHD-related behaviors with an OR of 2.3 (95% CI: 1.5, 3.8). These results were adjusted for prenatal and current household smoking exposure, sex, age, race/ethnicity, income to poverty ratio, preschool attendance, maternal age, and birth weight. A similar OR was estimated in analyses restricting the third tertile to children with concurrent blood Pb levels 1.3-5.0 µg/dL. The strongest association was observed in children with both high blood Pb level and prenatal tobacco smoke exposure. Compared to children with blood Pb levels <0.8 µg/dL and no prenatal exposure to tobacco smoke, children with blood Pb levels >1.3 µg/dL and prenatal exposure to tobacco smoking had the highest odds of parentally-rated ADHD-related behavior (OR: 8.1 [95% CI: 3.5, 18.7]). Although ADHD-related behavior was associated with low concurrent blood Pb levels (1.3-5 µg/dL), the contribution of higher past Pb exposures of adolescents born in the late 1980s cannot be excluded. Roy et al. (2009) also found an association with teacher ratings of ADHD-related behaviors using DSM criteria in children in Chennai, India; however, the study population included some very young children (i.e., age 3 years) and had relatively high concurrent blood Pb levels (mean: 11.4 µg/dL).

Other recent cross-sectional studies found concurrent blood Pb-associated higher ratings of ADHD-related behaviors using instruments that do not follow DSM criteria. Among children ages 8-11 years in Korea, Cho et al. (2010) found a stronger relationship with a total ADHD-related behaviors index as rated by teachers than parents. Mean ADHD ratings by teacher and parents were similar (both 9.1); however, parental ratings had greater variability (SD: 11.5 for parents and 8.6 for teachers), which could have contributed to differences in association. Among children in Romania, concurrent blood Pb level was associated similarly with parent and teacher ratings of ADHD-related behaviors (Nicolescu et al., 2010). As with individual behaviors described in preceding sections, blood Al and Hg levels were not associated with ratings of ADHD-related behaviors. Based on a log-linear model, a 1 µg/dL increase in concurrent blood Pb level within the 10th-90th percentile interval (1.8-7.1 µg/dL) was associated with a 5.9% increase (95% CI: 0, 13.7) in teacher rating of ADHD-related behavior. The association did not change substantially in an analysis that excluded the 5 children with blood Pb levels ≥ 10 µg/dL.

Attention Deficit Hyperactivity Disorder in Children

The 2006 Pb AQCD (U.S. EPA, 2006b) did not review studies of prevalence or incidence of ADHD diagnosis but noted lack of conclusive evidence for the effect of Pb exposure on ADHD based on a few small studies comparing blood or urine Pb levels between children with and without hyperactivity as identified by parents, teachers, or schools

(Gittleman and Eskenazi, 1983; David et al., 1972). As described in the preceding section, several recent cross-sectional studies found associations of concurrent blood Pb level with parent and teacher ratings of a total ADHD index, a composite index of attention, impulsivity, and hyperactivity. Results from a small body of recent studies also indicate associations of higher concurrent blood Pb level with prevalence of diagnosed ADHD in children ages 4-17 years (Nigg et al., 2010; 2008; Wang et al., 2008d; Braun et al., 2006). All of the studies were cross-sectional; thus, the temporal sequence between Pb exposure and ADHD incidence cannot be established. Further, while there are diagnostic guidelines for ADHD, the exact criteria or specific behaviors required can vary. Thus, within studies, there may be variations among subjects in the types of behaviors they displayed that led to a diagnosis of ADHD. While there is coherence with evidence from prospective studies in other populations for associations of Pb biomarker levels with characteristic behaviors, evidence specifically for ADHD prevalence was emphasized less than evidence for individual behaviors in drawing conclusions about the effects of Pb exposure on attention, hyperactivity, and impulsivity.

Associations between concurrent blood Pb level and ADHD prevalence were found in case-control studies conducted in different populations of children. While a limitation of these studies is potential selection bias arising from the nonrandom population sample, a common strength is their independent diagnosis of ADHD in a structured manner using parental and teacher ratings of behavior followed by independent assessment by multiple clinicians using DSM-IV criteria (Nigg et al., 2010; 2008; Wang et al., 2008d). Nigg et al. (2010; 2008) found an association between concurrent blood Pb level and ADHD diagnosis in relatively small (n = 150, 236) groups of children ages 6-17 years from the same community, with controls selected from healthy children who responded to the same mailings, community advertisements, or outreach to local clinics as cases. Wang et al. (2008d) found an association in a larger (n = 1,260) group of children in China, with controls selected from children admitted as outpatients to the same two pediatric clinics for respiratory infections.

Braun et al. (2006) found an association in children ages 4-15 years participating in NHANES 1999-2002. ADHD was ascertained by parent-report of ADHD diagnosis or use of stimulant medication, which is subject to reporting bias; however, the examination of multiple risk factors and outcomes in NHANES reduces the likelihood of biased participation and reporting of ADHD by parents of children specifically with higher Pb exposure. NHANES is not a random sample, but a strength over other studies that examined the prevalence of ADHD diagnosis is the large (n = 4,704) sample size and the nationally-representative results produced with adjustment for sampling weights in models. Surveillance data indicate that states with a higher percentage of children with blood Pb levels ≥ 10 µg/dL have lower prevalence of diagnosed ADHD (CDC, 2012,

2011c). These data do not support the potential for positive confounding of associations observed in the NHANES population by regional differences in blood Pb levels and prevalence of ADHD diagnosis. However, there is the potential for attenuation of associations with blood Pb levels if adjustment is not made for region.

With respect to blood Pb levels associated with ADHD diagnosis, analyses of the concentration-response relationship indicated monotonic increases in ORs across blood Pb level groups (Wang et al., 2008d; Braun et al., 2006). In the analysis of children in NHANES ages 4-15 years, compared to children with concurrent blood Pb level <0.8 µg/dL (7% below detection limit of 0.3 µg/dL), children with concurrent blood Pb level >2.0 µg/dL (maximum not reported) had higher prevalence of ADHD with an OR of 4.1 (95% CI: 1.2, 14.0). A similar OR was estimated for children with blood Pb levels 2.0-5.0 µg/dL (Braun et al., 2006). In the study of children in China, the highest OR was found in children ages 4-12 years with concurrent blood Pb levels ≥ 10 µg/dL but also was elevated in the group with blood Pb levels 5-10 µg/dL (OR: 4.92 [95% CI: 3.47, 6.98] compared with children with blood Pb level <5 µg/dL) (Wang et al., 2008d). Similar to findings in NHANES, Nigg et al. (2010; 2008) found associations at relatively low concurrent blood Pb levels, i.e., population means ~1 µg/dL (1.3%, 0% below detection limit of 0.3 µg/dL). However, because adolescents were examined, higher past Pb exposures could have contributed to the observed associations. Blood Pb levels are higher in early childhood, and among children participating in NHANES who were born 1984-1998, some likely had higher early-life Pb exposures as a results of the use of leaded gasoline in the U.S. (Braun et al., 2006).

Consideration for potential confounding varied among studies. In three-way analyses of covariance, Nigg et al. (2008) adjusted for sex and household income, and Nigg et al. (2010) adjusted for maternal IQ and prenatal smoking exposure. However, in preliminary analyses, Nigg et al. (2010) considered blood hemoglobin, household income, age, sex, and maternal smoking as potential confounding factors. The analysis of children participating in NHANES adjusted for age, sex, race, prenatal smoking exposure, postnatal smoker in the home, preschool/child care attendance, health insurance coverage, and ferritin levels but initially considered poverty to income ratio, birth weight, and admission to the neonatal intensive care unit (Braun et al., 2006). The results for children in China were adjusted for similar covariates and also family (parent and sibling) history of ADHD diagnosis, ascertained from clinical records (Wang et al., 2008d). Family history of ADHD was selected as a covariate based on its association with child ADHD; no information was provided on its association with child blood Pb level. None of the studies of ADHD prevalence considered potential confounding by current parental caregiving quality.

4.3.3.2 Conduct Disorders

Epidemiologic Studies of Conduct Disorders in Children

As described in the introduction to externalizing behaviors, there are two domains of conduct disorders: undersocialized aggressive conduct disorder and socialized aggressive conduct disorder (as reviewed inWhitcomb and Merrell, 2012). In this evaluation of a relationship with Pb exposure, the two domains are combined because they cannot be easily distinguished in the available epidemiologic studies that combine various behaviors into a composite index, find associations with behaviors within each domain, or examine outcomes characterized by behaviors in each domain. This section also evaluates the evidence for criminal offenses which can be predicted by earlier conduct disorders (Soderstrom et al., 2004; Babinski et al., 1999; Pajer, 1998).

The 2006 Pb AQCD (U.S. EPA, 2006b) described several prospective studies that found associations of higher blood, tooth, or bone Pb levels with behaviors related to conduct disorders in children as rated by parents or teachers or criminal offenses in adolescents (also see Table 4-12). Supporting evidence from recent prospective studies included follow-up of previous cohorts to older ages. Recent cross-sectional studies found associations between concurrent blood Pb level and ratings of behaviors related to conduct disorders, but aside from difficulty in establishing temporality, these recent cross-sectional studies were limited by their less extensive consideration for potential confounding.

Table 4-12 Associations between Pb biomarker levels and behaviors related to conduct disorders in children and young adults.

Study	Study Population and Methodological Details Organized by outcome category. Within each category, results are grouped by strength of study methodology, generalizability[a]	Blood (µg/dL), Tooth, or Bone (µg/g) Pb Metrics Analyzed	Outcomes Analyzed	Effect Estimate (95% CI)[b]
Prospective Studies of Behavioral Ratings				
Dietrich et al. (2001)	186 children followed prenatally (1979-1985) to ages 15-17 yr, Cincinnati, OH Recruitment at prenatal clinic. Moderate follow-up participation but no selective attrition. Mostly African-American. Linear regression model adjusted for HOME, parental IQ, current SES, birth weight. Also considered potential confounding by maternal age, prenatal smoking, alcohol use, and marijuana use, other birth outcomes, ear infections, Fe status, sex, age, caregiver education, public assistance, preschool attendance, # children and adults in home.	0-6 yr avg blood Median (5th-95th): 12 (6.0-26) See Wright et al. (2008)	Self-Report of Delinquent Behavior Score Parental Report of Predelinquent and Delinquent Behavior Score Ages 15-17 yr	0.10 (0.01, 0.19) 0.09 (-0.02, 0.20)
Burns et al. (1999)	322 children followed from birth (1979-1982) to ages 11-13 yr, Port Pirie, Australia. Moderate follow-up participation. Participants had higher birth weight, older mothers, less educated fathers. Log linear regression model adjusted for maternal age, prenatal smoking status, IQ, concurrent psychopathology, and education, birth weight, type of feeding, length of breastfeeding, paternal education and occupation, birth order, family functioning, parental smoking, marital status, HOME score, child IQ.	Lifetime (age 11-13 yr) avg blood GM (5th-95th) Boys 14.3 (13.5-15.1) Girls: 13.9 (13.2-14.6) Intervals analyzed (10th-90th percentiles) Boys: 13.7-14.9 Girls: 13.4-14.5	Aggressive Score, boys Aggressive Score, girls Delinquent score, boys Delinquent score, girls Maternal rating by Child Behavior Checklist at ages 11-13 yr	0.17 (0.08, 0.26) 0.10 (0, 0.21) 0.06 (0.02, 0.09) 0.01 (-0.01, 0.04)

Table 4-12 (Continued): Associations between Pb biomarker levels and behaviors related to conduct disorders in children and young adults.

Study	Study Population and Methodological Details Organized by outcome category. Within each category, results are grouped by strength of study methodology, generalizability[a]	Blood (µg/dL), Tooth, or Bone (µg/g) Pb Metrics Analyzed	Outcomes Analyzed	Effect Estimate (95% CI)[b]
Chandramouli et al. (2009)	488 children followed from age 4 mo (born 1992) to ages 8 yr, Avon, U.K. All births in area eligible. Similar characteristics as U.K. census, high participation at baseline and follow-up. Participants had better educated mothers, who smoked less, better home environment. Log linear regression model adjusted for maternal education and smoking, home ownership, home facilities score, family adversity index, paternal SES, parenting attitudes at 6 mo, child sex. Also considered potential confounding by child IQ.	Age 30 mo blood Mean (SD): NR	Antisocial activities Parent or teacher rating by Antisocial Behaviour Interview at age 8 yr	ORs for increased score
		Group 1: 0-<2		Reference
		Group 2: 2-<5		0.93 (0.47, 1.83)[c]
		Group 3: 5-<10		1.44 (0.73, 2.84)[c]
		Group 4: >10		2.90 (1.05, 8.03)[c]
Needleman et al. (1996)	212 boys selected from prospective cohort followed from 1st grade to ages 11-14 yr, Pittsburgh, PA Nested case-control. Low follow-up participation. Participants had higher SES, lower maternal IQ, smaller family size, higher IQ, were nonwhite. ANCOVA with behavior scores adjusted for maternal age, IQ, occupation, and education, presence of both parents in home, # children in family, race, history of medical problems, age, score at age 7 yr. Did not consider potential confounding by parental caregiving quality.	Bone at ages 9-14 yr: NR High bone Pb: upper bound of quintile 3 and higher Low bone Pb: rest of quintile 3 and lower Exact levels NR	Delinquent score (square root)	Low Pb: 1.18 High Pb: 1.45, p=0.04
			Aggressive score (square root) Parent rating by Child Behavior Checklist at age 11-14 yr	Low Pb: 2.43 High Pb: 2.98, p=0.009
Wasserman et al. (2001)	191 children followed prenatally (1984-1985) to ages 4-5 yr, Pristina, Yugoslavia. Recruitment from prenatal clinics. High follow-up participation, participants had less educated mothers, higher average blood Pb, were Albanian. Log linear regression model adjusted for sex, ethnicity, age, maternal education and smoking history, HOME score, birth weight.	Lifetime (to age 4-5 yr) avg blood Mean of log= 0.86, Mean ~7.2	Aggressive Score Delinquent Score Maternal rating by Child Behavior Checklist at ages 4-5 yr	Per log increase: 0.08 (-0.19, 0.35) 0.32 (0.03, 0.61)

Table 4-12 (Continued): Associations between Pb biomarker levels and behaviors related to conduct disorders in children and young adults.

Study	Study Population and Methodological Details Organized by outcome category. Within each category, results are grouped by strength of study methodology, generalizability[a]	Blood (µg/dL), Tooth, or Bone (µg/g) Pb Metrics Analyzed	Outcomes Analyzed	Effect Estimate (95% CI)[b]
Bellinger et al. (1994b)	1,782 children followed from birth (1979-1980) to age 8 yr, Boston, MA area Recruitment at birth hospital. Moderate follow-up participation. More participants were white, had lower cord blood Pb levels, better birth outcomes. Log linear regression model adjusted for prepregnant weight, race, Cesarean section, maternal marital status, prenatal care, paternal education, colic, child current medication use, sibship size, sex, birth weight. Also considered potential confounding by public assistance, prenatal smoking, maternal education but not parental caregiving quality.	Tooth (age 6 yr) Mean (SD): 3.4 (2.4) Interval analyzed: 1.2-6.3 = 10th-90th percentiles	Total externalizing behavior T score, (inattentive, nervous-overactive, aggressive) Teacher rating by Child Behavior Profile at age 8 yr	0.51 (0.21, 0.81)
Chen et al. (2007)	721 children participating in TLC trial, followed from age 1-3 to 5 yr, Baltimore, MD; Cincinnati, OH; Newark, NJ; Philadelphia, PA. Multi-city, high participation rate. Eligible for chelation trial because of high age 1-3 yr blood Pb levels (20-44 µg/dL). Linear regression-based path analysis adjusted for city, race, sex, language, single parent, parental education and employment, age at blood Pb measurement, caregiver IQ. Considered potential confounding by chelation treatment but not parental caregiving quality. Direct = independent of IQ. Indirect = mediated through IQ.	Age 2 year Mean (SD): 26 (5.1) Interval analyzed: 20-33 =10th-90th percentiles	Oppositional Index Score Direct Indirect	 0.12 (-0.08, 0.31) 0.04 (0.002, 0.07)
		Concurrent Mean (SD): 12 (5.2) Interval analyzed: 6.6 (10th percentile)-10	Direct Indirect	0.12 (-0.08, 0.32) 0.05 (-0.03, 0.13)
Cross-sectional studies of Behavioral Ratings				
Braun et al. (2008)	2,867 children ages 8-15 yr (born 1986-1996), U.S. NHANES 2001-2004 Large multi-location study of multiple risk factors and outcomes. Subjects with available data were older, white, higher SES, with lower blood Pb levels, higher birth weight, and fewer had household smokers. Logistic regression model adjusted for child age, sex, and race, poverty income ratio, maternal age, prenatal smoke exposure, cotinine levels. Did not consider potential confounding by parental caregiving quality.	Concurrent blood Q1: 0.2-0.7 Q2: 0.8-1.0 Q3: 1.1-1.4 Q4: 1.5-10 Detection limit = 0.3	Conduct disorder Parental report using Diagnostic Interview Schedule for Children-Caregiver Module at ages 8-15 yr	ORs (yes/no) Q1: Reference Q2: 7.2 (1.1, 49)[c] Q3: 12 (2.4, 65)[c] Q4: 8.6 (1.9, 40)[c]

Table 4-12 (Continued): Associations between Pb biomarker levels and behaviors related to conduct disorders in children and young adults.

Study	Study Population and Methodological Details Organized by outcome category. Within each category, results are grouped by strength of study methodology, generalizability[a]	Blood (µg/dL), Tooth, or Bone (µg/g) Pb Metrics Analyzed	Outcomes Analyzed	Effect Estimate (95% CI)[b]
Chiodo et al. (2007)	452-460 African-American children, age 7 yr (born 1989-1991), Detroit, MI area Recruitment at prenatal clinic. High prevalence prenatal drug exposure. High participation rate. Linear regression model adjusted for sex (both outcomes), caretaker education, HOME, maternal prenatal alcohol use, current marijuana use (delinquent behavior), maternal age, # children in home (social problems). Also considered potential confounding by SES, child age, maternal prenatal and current drug and alcohol use, and IQ, caretaker current psychopathology.	Concurrent blood Mean (SD): 5.0 (3.0) Interval analyzed: 2.1-8.7 = 10th-90th percentiles	Delinquent behavior Social problems Teacher rating by Achenbach Teacher Report Form at age 7 yr	0.09 (0, 0.18)[d] 0.10 (0, 0.20)[d]
Sciarillo et al. (1992)	201 children (born 1984-1987) ages 2-5 yr, Baltimore, MD. Convenience sample. No information on participation rate. 100% African-American. Linear regression adjusted for maternal education, employment status, marital status, current depressive symptom score, # preschool children in the home, child age, sex, and Fe deficiency.	Concurrent blood Mean (SD) Low: 9.2 (2.9) High: 28 (10) Interval analyzed: 5.9 (10th percentile of low group)-10	Total Behavioral Problem score (aggressive, destructive, somatic problems, sleep problems, depressed, social withdrawal, etc) Maternal rating by Child Behavior Checklist at ages 2-5 yr	0.18 (0.04, 0.32)
Nigg et al. (2008)	150 children, ages 8-17 yr, birth yr and location NR. Case-control study of ADHD. Recruitment by community advertisements, mailings, outreach to clinics. Moderate participation rate from eligibles. Could have biased participation by Pb exposure leading to biased behavioral ratings. Did not consider potential confounding factors.	Concurrent blood Mean (SD): 1.03 (0.49) Detection limit=0.3	Oppositional defiant disorder Index Parent, teacher rating Conners Rating Scale-Revised	Pearson r = 0.18, p <0.05
Studies of Criminal Offenses				
Wright et al. (2008)	250 adults followed from birth (1979-1984) to age 19-24 yr, Cincinnati, OH Prospective. Recruitment at prenatal clinic. 90% African-American. Moderate follow-up participation. No selective attrition. Negative binomial regression model adjusted for maternal IQ and education, sex, SES. Also considered potential confounding by maternal prenatal smoking, marijuana use, narcotic use, and prior arrests, HOME score, birth weight, # children in the home, public assistance in childhood.	Age 6 yr blood Pb Median (5th-95th): 6.8 (3.4-18) Age 0-6 yr avg Median (5th-95th): 12 (6.0-26)	Criminal arrests From county records at ages 19-24 yr Age 6 yr Age 0-6 avg	RRs (yes/no): 1.05 (1.01, 1.09) 1.01 (0.98, 1.05)

Table 4-12 (Continued): Associations between Pb biomarker levels and behaviors related to conduct disorders in children and young adults.

Study	Study Population and Methodological Details Organized by outcome category. Within each category, results are grouped by strength of study methodology, generalizability[a]	Blood (µg/dL), Tooth, or Bone (µg/g) Pb Metrics Analyzed	Outcomes Analyzed	Effect Estimate (95% CI)[b]
Fergusson et al. (2008)	853 children followed from birth (1977) to age 21 yr, Christchurch, New Zealand Prospective. High follow-up participation. Participants had lower SES. Negative binomial regression model adjusted for maternal education and prenatal cigarettes/ day, ethnicity, family conflict, physical abuse in childhood. Also considered potential confounding by traffic density in childhood, maternal age, paternal education, average family income, maternal use of punishment, parental drug use, parental bonding, child marijuana use, parent alcohol problems.	Tooth (age 6-8 yr) Mean: 6.2	Number convictions for property or violent offenses From police records at ages 14-21 yr	0.20 (0, 0.40)
Needleman et al. (2002)	340 adolescents, ages 12-18 yr (born 1978-1986), Pittsburgh, PA area. Case-control. 194 cases (county program) and 146 controls (Pittsburgh high schools) from different sources. Low participation from cases. Logistic regression adjusted for race, parental education and occupation, both parents in home, neighborhood crime rate, #children in home. Did not consider potential confounding by parental caregiving quality.	Concurrent Bone Mean (SD) in ppm Cases: 11 (33) Controls: 1.5 (32)	Delinquent status From Juvenile Court records at ages 12-18 yr	ORs (yes/no) for bone Pb level >25 ppm White: 3.8 (1.1, 13)[c] African-American: 2.2 (0.5, 10.0)[c]

[a]Results are organized by outcome category, behavior ratings then documented criminal offenses. Within each category, studies are grouped by strength of study design, representativeness of the study population characteristics and Pb biomarker levels examined, and extent of consideration for potential confounding. There is not necessarily a continuum of decreasing strength across studies.

[b]Unless otherwise specified, effect estimates are standardized to a 1 µg/dL increase in blood Pb level or 1 µg/g increase in bone or tooth Pb level in the interval from the 10th percentile to 10 µg/dL blood Pb or 10 µg/g tooth or bone Pb or the 90th percentile, whichever is lower.

[c]Odds in higher quantile of blood or bone Pb level compared to that in lowest quantile of blood or bone Pb level (reference).

[d]95% CI was estimated from a reported p-value of 0.05.

Collectively, the evidence from prospective studies indicated associations of teacher and parental ratings of aggressive, antisocial, and delinquent behavior with biomarkers of cumulative Pb exposure, i.e., age 0-6 year average blood, lifetime (to ages 4-5 or 11-13 years) average blood, tooth, and bone Pb level. Associations with tooth (Bellinger et al., 1994b) and bone (Needleman et al., 1996) Pb level, collected prior to or at the same time as behavioral assessments, respectively, were found with adjustment for several potential confounding factors as noted above, with the exception of parental caregiving quality. Collective evidence from prospective studies indicated associations between blood Pb level and ratings of behaviors related to conduct problems with population mean blood Pb levels 6.8-14.3 µg/dL (prenatal maternal, age 6 year, lifetime average to 4-5 or 11-13 years) (Dietrich et al., 2001; Wasserman et al., 2001; Burns et al., 1999). In a study of children ages 7-8 years in the U.K. born in the 1990s, Chandramouli et al. (2009) added to the evidence for associations at relatively high blood Pb levels by finding that compared with children ages 8 years with age 30 month blood Pb levels 0-2 µg/dL, children with blood Pb levels >10 µg/dL had increased odds of greater antisocial activities as rated by parents or teachers with an OR of 2.9 (95% CI: 1.1, 8.0). The Boston cohort had lower childhood blood Pb levels (seeBellinger et al., 1992); however, tooth Pb level was mostly strongly associated with a higher total externalizing behavior score, which also included attention, overactive/nervous, and aggression (Bellinger et al., 1994b). Weaker associations were found specifically with aggressive or destructive behavior.

As described above, cross-sectional studies also indicated blood Pb-associated higher ratings of conduct disorders. Associations were found with lower blood Pb levels than in prospective studies, concurrent means 1 and 5 µg/dL (ages 7-17 years). However, for these children, the influence of higher Pb exposures earlier in childhood cannot be excluded. Further, because of their various limitations discussed below, cross-sectional results were less of a consideration in drawing conclusions about the effects of Pb exposure on conduct disorders. In the recent analysis of 2,867 children ages 8-15 years participating in NHANES 2001-2004, Braun et al. (2008) analyzed blood Pb level as a categorical variable and found higher prevalence of conduct disorder as ascertained by parents (Diagnostic Interview Schedule for Children which follows DSM-IV criteria) with concurrent blood Pb levels in the range of 0.8 to 1.0 µg/dL. Compared with children with blood Pb levels 0.2-0.7 µg/dL (6% below detection limit of 0.3 µg/dL), the OR in children with blood Pb levels 0.8-1.0 µg/dL was 7.2 (95% CI: 1.1, 49). The wide 95% CIs could have resulted from the small numbers of cases of conduct disorder. For example, there were 22 children rated as having conduct disorder in the group with blood Pb levels 0.8-1.0 µg/dL. Nigg et al. (2008) found a blood Pb-associated higher rating (parent or teacher) of oppositional defiant disorder in a population with similarly low concurrent blood Pb levels, means ~1 µg/dL. However, the implications are limited

because of the ADHD case-control design (Section 4.3.3.1) and lack of consideration of potential confounding factors. Further, because both studies examined adolescents who likely had higher earlier childhood Pb exposures, there is uncertainty regarding the level, timing, frequency, and duration of Pb exposure that contributed to the observed associations.

Consideration for potential confounding varied among the cross-sectional studies of behaviors related to conduct disorders. With the exception of Nigg et al. (2008), many considered sex, race, SES, and parental education. However, only a few considered parental caregiving quality (i.e., HOME score). An analysis of children in Baltimore, MD adjusted results for child Fe deficiency but examined children ages 2-5 years and analyzed a total behavior problem score, which included various externalizing and internalizing behaviors (Sciarillo et al., 1992). Chiodo et al. (2007) found that higher concurrent blood Pb level was associated with higher teacher ratings of social problems and delinquent behavior in children ages 7 years in Detroit, MI with consideration for HOME score. The study population had high prevalence of prenatal drug exposure. Drug exposure did not meet the criteria for inclusion in the model, indicating lack of confounding by this factor. While prenatal drug exposure may not affect internal validity of the results, it may limit the generalizability of the results to the general U.S. population of children. Lack of representativeness also may pertain to the results of Chen et al. (2007), who examined children participating in a chelation trial because of high blood Pb levels (20-44 μg/dL) at ages 1-3 years.

While few in number, evidence from prospective studies also indicated associations of biomarkers of earlier childhood Pb exposure with delinquent and criminal acts as objectively assessed from government records. These studies of delinquent and criminal acts examined Pb levels in blood or tooth samples collected in the 1980s when Pb exposures were much higher than those of the current U.S. population (Fergusson et al., 2008; Wright et al., 2008). However, the prospective study design and consideration for several potential confounding factors increase confidence that the observed associations represent a relationship with Pb exposure.

In the Cincinnati cohort, prenatal maternal 1st trimester, age 6 year, and age 0-6 year average blood Pb levels were associated with self- and parent-reported delinquent and antisocial acts at ages 15-17 years (Dietrich et al., 2001). Wright et al. (2008) recently extended these findings to include associations with criminal and violent criminal arrests at ages 19-24 years. In models that adjusted for maternal IQ and education, sex, and SES, the relative risks (RRs) for total arrests per 1 μg/dL increment in blood Pb level were 1.07 (95% CI: 1.01, 1.13) for prenatal blood Pb level, 1.01 (95% CI: 0.97, 1.05) for age 0-6 year average blood Pb level, and 1.05 (95% CI: 1.01, 1.09) for blood Pb level at age

6 years. Interactions terms for blood Pb by sex were not statistically significant; however, the attributable risk was considerably higher for males (0.85 arrests/year [95% CI: 0.48, 1.47] for age 6 year blood Pb) than females (0.18 [95% CI: 0.09, 0.33]). A strength of Wright et al. (2008) was the detailed examination of potential confounding by a large number of variables (Table 4-12). All of the examined covariates were weakly correlated with blood Pb levels (r = 0.24-0.35), thereby reducing the potential for confounding by the examined factors. Nonetheless, variables such as maternal IQ and education, sex, and SES were included in the model because they were associated with $p \leq 0.05$ with arrests in the full multivariate model or changed the blood Pb level estimate by more than 10%. HOME score was similar between subjects with and without criminal arrest records and did not meet the criteria for inclusion in final models.

The study of the New Zealand cohort also considered several potential confounding factors including several related to SES, family functioning, and parental bonding (Table 4-12) (Fergusson et al., 2008). Per log increase in Pb in teeth obtained between ages 6 and 8 years, there was a 0.49 (95% CI: 0.16, 0.82) increase in the number of documented violent or property convictions at ages 14-21 years. Results were adjusted for SES, ethnicity, parental criminal offenses, and parental alcoholism. The effect estimate for tooth Pb level decreased in adjusted models and was found to account for <1% in the variance of criminal convictions; however, the association remained statistically significant.

The epidemiologic studies described above varied in study design, examination of specific behaviors related to conduct disorders, and method of assessment. The consistency of association of Pb biomarker levels with conduct disorders was corroborated in a recent meta-analysis (Marcus et al., 2010) that included 19 studies (several of which are described above) with a total of 8,561 children and adolescents (range of mean ages: 3.5-18.4 years). Effect estimates were converted to Pearson correlation coefficients, and the combined effect estimate was r = 0.19 (95% CI: 0.14, 0.23). The key finding of this study was the robustness of association to many between-study sources of heterogeneity. In the meta-analysis, effect sizes did not significantly differ by population mean blood Pb level, by prospective or cross-sectional design, by the various measures of conduct disorders examined (i.e., oppositional behavior, delinquency, externalizing problem composite measure), or by outcomes assessed using self-report, parent report, teacher report, or criminal records. Adjustment for covariates such as SES, birth weight, parental IQ, and home environment did not attenuate the relationship between Pb biomarker level and conduct disorders. In addition to strengthening the evidence for the independent associations of Pb biomarker levels with conduct disorders, the results indicated that the lack of adjustment for any particular covariate, including HOME score, does not warrant limiting inferences from a particular

study. The major source of heterogeneity in effect estimates was the biomarker of Pb examined. A larger magnitude of effect was estimated for hair Pb levels compared with bone, tooth, or blood Pb levels, which had similar effect sizes. Hair Pb has been purported to be a better indicator of cumulative Pb exposure compared to bone Pb levels due to the high turnover of bone throughout childhood and into adolescence (Marcus et al., 2010); however, an empirical basis for interpreting hair Pb measurements in terms of Pb body burden or exposure has not been firmly established (Section 3.3.4.2).

Several studies of conduct disorders aimed to characterize whether associations with Pb biomarkers were independent of effects on cognitive function. These studies found that associations of Pb biomarkers with conduct disorders remained statistically significant in a model that additionally adjusted for child IQ or academic achievement, indicating that Pb exposure may have a direct effect on conduct disorders independent of its effect on cognitive function (Chandramouli et al., 2009; Fergusson et al., 2008; Burns et al., 1999). However, simple statistical adjustment for cognitive function indices may underestimate an effect of Pb on conduct disorders because a decrement in cognitive function may lie on the causal pathway to behavioral problems. Chen et al. (2007) used path analysis to characterize the direct effects and indirect effects (mediated/occurring through child IQ) of blood Pb level on an oppositional index at age 5 years and total externalizing behavior ratings at age 7 years. There was evidence of a direct effect of Pb exposure, particularly for total externalizing behavior ratings at age 7 years (Table 4-12). These findings may have limited applicability to the general U.S. population given that some children in the study had been given chelators at ages 1-3 years because of high blood levels, and it is possible that the observed associations were due to the residual effect of high earlier childhood blood Pb levels (20-44 µg/dL).

Toxicological Studies of Aggression

While recent studies were not identified, evidence available in the 2006 Pb AQCD (U.S. EPA, 2006b) pointed to the effects of Pb on changes in social behavior of rodents and nonhuman primates. Most observations comprised Pb-induced increases in social and sexual investigation, as indicated by sniffing, grooming, following, mounting, and lordosis behavior. In animals, the social behavior most comparable to findings for conduct disorders in children is aggression; however, the effects of Pb on aggression in animals were inconsistent. In animals, aggression was assessed as threats, attacks, bites, chases, and offensive posture in encounters with other animals, and Pb exposure was found to not affect, decrease, and increase aggression. Pb exposure generally was not found to affect aggression in juvenile animals; however, increased aggression was found in adult animals with high concentration (>2,500 ppm) gestational plus postnatal dietary Pb exposure.

Delville (1999) found Pb-induced increases in aggression with the lowest concentration Pb exposure examined among all animal studies of aggression. Golden hamsters exposed to 100 ppm Pb acetate GD8-PND42 in drinking water had blood Pb levels of 10 to 15 μg/dL at PND42. As adults at PND45, Pb-exposed animals displayed more aggression as measured by attacking and biting an intruder put in the cage. In mice, higher Pb exposure produced mixed findings. BK:W mice exposed to 1,300 ppm Pb acetate in drinking water from gestation through age 18 weeks displayed increased social and sexual investigation but not aggression (femur Pb level at 34 weeks: 5,364 μM Pb/g ash in males, 4,026 μM Pb/g ash in females) (Donald et al., 1986). In another study from the same laboratory, BK:W mice were exposed to 2,500 ppm Pb acetate in drinking water from gestation through age 17-18 weeks, and found shorter latencies to aggression in Pb-exposed male mice than in controls (Donald et al., 1987). In juvenile Long-Evans hooded rats, lactation-only (PND1-PND21) exposure to 670 ppm Pb chloride in drinking water increased "rough and tumble" play behavior at PND26 which was not characterized as aggression because of the lack of injury, submissive posturing, or escape attempts in encounters with other animals (Holloway and Thor, 1987).

4.3.3.3 Integrated Summary of Externalizing Behaviors

Attention, Impulsivity, and Hyperactivity

Attention, impulsivity, and hyperactivity are included within the attention deficit hyperactivity disorder domain of externalizing behaviors. Although not as extensively as cognitive function, several epidemiologic studies have examined the relationship between Pb exposure in children and attention, impulsivity, and hyperactivity in children and young adults. A majority has examined attention, and some of these studies also examined impulsivity or hyperactivity. Few studies have examined ratings of a composite index of ADHD-related behaviors or ADHD diagnosis and are not the focus of this evaluation. The focus of the evaluation is on evidence for attention but draws on coherence with evidence for impulsivity and hyperactivity, evidence in animals, and that describing potential modes of action. The collective epidemiologic evidence base for attention comprises many prospective studies, which also were reviewed in the 2006 Pb AQCD (U.S. EPA, 2006b), as well as many cross-sectional studies, some reviewed previously and some recently published. Whether prospective or cross-sectional or previously reviewed or recently published, most of the epidemiologic studies found associations between childhood blood or tooth Pb levels and attention decrements, impulsivity, and hyperactivity (Figure 4-9 and Table 4-11). Not all results were

statistically significant, but results mostly showed a pattern of attention decrements, impulsivity, and hyperactivity with higher blood or tooth Pb level.

Whether prospective or cross-sectional, most studies had population-based recruitment from prenatal clinics, hospitals at birth, or schools and had moderate to high participation. A few prospective studies had increased loss-to-follow-up in certain groups, for example, lower SES, lower earlier FSIQ, lower HOME score. This potential selection bias can reduce the generalizability of findings to the original study population, but there was not strong indication that participation was biased to those with higher blood Pb levels and greater attention decrements, impulsivity, or hyperactivity. Multiple testing was common; however, in most studies, the consistent pattern of association observed across the ages of blood Pb level and/or behavior examined increases confidence that the evidence is not unduly biased by the increased probability of finding associations by chance alone.

With analysis of prenatal, earlier childhood (e.g., age 6 year) or lifetime average (e.g., to ages 4-5 years or 11-13 years) blood or tooth Pb levels and measures of attention from later in childhood between ages 4 and 20 years, the prospective studies better characterized the temporal sequence between exposure and outcome. In prospective studies, associations of blood or tooth Pb levels with attention were found in diverse populations ages 8-20 years in North America, Australia, and New Zealand. These prospective studies examined populations with higher blood Pb levels (means 7-14 µg/dL) than did cross-sectional studies. Prospective studies found associations of blood and tooth Pb levels with attention as assessed using neuropsychological tests or ratings by parents or teachers. Observations of associations across the various methods of assessment increase confidence that the collective evidence is not unduly influenced by lower attention ratings biased to parents of children with higher blood Pb levels or Pb exposures. Prospective studies that examined attention with the CPT found associations with blood or tooth Pb level. Increases in commission and omission errors or reaction time were associated with higher prenatal (maternal) and earlier childhood (age 3-60 month average, age 78 month) blood Pb levels in the Cincinnati cohort at ages 15-17 years (Ris et al., 2004) and with higher tooth Pb (from 1st/2nd grade) levels in Boston-area young adults at ages 19-20 years (Bellinger et al., 1994a). Results from prospective studies also indicated lower parent and teacher ratings of attention in association with higher lifetime average blood Pb levels in children ages 11-13 years in Port Pirie, Australia (Burns et al., 1999) and with tooth Pb levels (from ages 6-8 years) in children ages 8-13 years in New Zealand, and Boston, MA (Fergusson et al., 1993; Leviton et al., 1993). The mean blood Pb levels across the populations examined were 6.8 and 8.3 µg/dL for prenatal (maternal or cord) blood, 13.4 for age 3-60 month average, and 14 µg/dL for lifetime average blood. A recent prospective study in the U.K. found that children ages 7-8 years with age 30 month blood Pb levels > 10 µg/dL had higher teacher

ratings of hyperactivity but not selective attention compared with children with blood Pb levels 0-2 µg/dL (Chandramouli et al., 2009). A null association with attention was found in the Yugoslavia cohort with a mean lifetime (to ages 4-5 years) blood Pb level of 7.2 µg/dL (Wasserman et al., 2001). However, behaviors may be less reliably measured in the young children ages 4-5 years.

In children, attention was associated with biomarkers of Pb exposure representing several different lifestages and time periods. Prospective studies did not examine a detailed Pb biomarker history, and results do not identify an individual critical lifestage, time period, or duration of Pb exposure associated with attention decrements in children. Associations in prospective studies for attention decrements with tooth Pb level, early childhood average and lifetime average blood Pb levels point to an effect of cumulative Pb exposure. Prospective studies did not examine indicators of more recent Pb exposure. A strength of the prospective studies was their more extensive consideration for potential confounding. Although the specific factors varied by study, prospective studies of attention adjusted for factors such as SES, parental IQ, maternal education, parental caregiving quality, self drug use, prenatal drug and alcohol exposure, and birth outcomes. As described in Section 4.3.3, a few studies have tried to assess potential confounding by parental psychopathology (Cho et al., 2010; Nicolescu et al., 2010; Silva et al., 1988) but with the use of parental self-report, which may be subject to measurement error. At present, there is not evidence to support parental psychopathology as a direct confounding factor, i.e., contributing to a spurious association with Pb biomarkers when there is none. However, parental psychopathology has been speculated to influence child Pb exposure via a potential relationship with parenting caregiving quality. A clear relationship between parental psychopathology and poorer parenting behavior has not been established (as reviewed in Johnston et al., 2012), and adjustment for parental psychopathology may control for Pb exposure itself. However, if there is a noncausal correlation between parental psychopathology and Pb exposure and it is mediated by (i.e., occurs through) parental caregiving quality, then direct adjustment for parental caregiving quality may help address potential confounding by parental psychopathology.

Most cross-sectional studies found attention decrements in children ages 3-16 years in association with concurrently measured blood or tooth Pb level. Among studies that examined multiple behaviors, most also found associations with impulsivity and/or hyperactivity. A limitation of cross-sectional associations with concurrent blood Pb levels is the greater uncertainty about the potential for reverse causation. The cross-sectional studies included a mix of previous and recent studies and a wide range of population mean blood Pb levels (1-11.5µg/dL). There also was a mix of preschool-aged and schoolaged children. Among studies of populations with relatively high concurrent blood Pb levels (means 11.1-13.2 µg/dL), most found that higher blood Pb levels were

associated with higher parent or teacher ratings of attention decrements and hyperactivity (Roy et al., 2009; Kordas et al., 2007; Silva et al., 1988). Higher tooth Pb levels also were associated with higher teacher ratings of hyperactivity in children in grades 1-3 (Rabinowitz et al., 1992; Needleman et al., 1979). Needleman et al. (1979) also found an association with impulsivity ratings and reaction time. These cross-sectional studies of higher blood Pb levels found associations with adjustment for SES, child age, sex, family structure, and parental education, but most did not examine parental caregiving quality.

Some cross-sectional studies examined lower blood Pb levels (means 1-6.5 µg/dL for ages 4-17 years) closer to those measured in most current U.S. children. There was heterogeneity in the extent of potential confounding and ages of children examined, which makes drawing conclusions difficult. Among the lower blood Pb level studies that had more extensive consideration for potential confounding, most found higher concurrent blood Pb levels to be associated with attention decrements and also with impulsivity or hyperactivity. All of these studies adjusted for or considered potential confounding by SES as well as parental IQ, education, and caregiving quality. In the Rochester cohort, concurrent blood Pb level (mean 6.5 µg/dL) was not associated strongly with attention as rated by study examiners (Canfield et al., 2003b). However, in the young children ages 4 years, behaviors may be less reliably measured. Also, in the Rochester cohort, the association with attention was attenuated only after adjustment for color/shape knowledge and child IQ. These inter-related outcomes may share some variance. In another study of younger children ages 5-6 years in Canada, higher concurrent blood Pb level was associated with higher examiner ratings of attention decrements, impulsivity, and hyperactivity (Plusquellec et al., 2010). Among older children (age 7 years) in Detroit, MI, higher concurrent blood Pb level (means: 5.0, 5.4 µg/dL) was associated with higher examiner or teacher ratings of attention decrements and hyperactivity (Chiodo et al., 2007; 2004). These studies also found associations with attention decrements as assessed by the CPT, although not strongly with all examined indices of the CPT. In these populations with high prevalence of prenatal alcohol or drug exposure, results did not indicate confounding by these other exposures. However, the presence of these other exposures may limit the generalizability of results. Further, in older children, the contribution of higher Pb exposures earlier in childhood cannot be excluded. While higher-quality cross-sectional evidence in populations with mean blood Pb levels 5-6.5 µg/dL show associations of blood Pb with attention decrements, impulsivity, and hyperactivity, there are limitations related to young age of subjects or generalizability of the study population.

Other cross-sectional studies examined even lower concurrent blood Pb levels, 1-3.7 µg/dL and found associations with attention, impulsivity, and hyperactivity in children ages 8-17 years (Cho et al., 2010; Nicolescu et al., 2010; Nigg et al., 2008). These

associations were found with parental or teacher ratings, with attention measured by the CPT, and impulsivity measured with response inhibition tests. Associations between blood Pb level and attention or impulsivity also were found with adjustment for child IQ (Cho et al., 2010; Nigg et al., 2008), supporting an effect of Pb exposure on these behaviors independent of effects on cognitive function. Despite finding associations with more objective measures of attention or impulsivity, a limitation of these studies was their less consistent consideration for potential confounding factors. Studies varied in adjusting results for SES and parental education, and none considered parental caregiving quality. A limitation specific to Nigg et al. (2008) is the case-control study design of children with and without ADHD could result in biased ratings of hyperactivity by blood Pb level if participation by children with ADHD is biased to those with higher Pb exposure.

Recent cross-sectional studies also found associations between higher concurrent blood Pb levels and higher parental ratings of a composite index of ADHD-related behaviors, including a large NHANES analysis that used DSM-IV criteria (Froehlich et al., 2009). In the few available studies, concurrent blood Pb levels were associated with prevalence of diagnosed ADHD in children (Section 4.3.3.1). There is coherence with evidence from prospective studies for associations of blood and tooth Pb levels with attention, hyperactivity, and impulsivity, which are behaviors evaluated in the diagnosis of ADHD. However, the small number of studies, their cross-sectional or case-control design, inconsistent adjustment for SES and parental education, and lack of consideration for potential confounding by parental caregiving quality preclude conclusions regarding the relationship between Pb exposure and ADHD specifically.

As described above, consideration of potential confounding factors varied particularly among cross-sectional studies. Regardless of study design, adjustment for SES is difficult as it is highly correlated with Pb exposure and there is no single measure that fully represents SES. Residual confounding also is likely by factors not considered or factors measured with error. However, in addition to evidence from prospective studies that considered several well-characterized potential confounding factors, the coherence with evidence that Pb exposure induces greater distractibility and impulsivity in animals increases confidence that the associations of blood and tooth Pb levels with attention decrements and impulsivity observed in children represent a relationship with Pb exposure. Evidence in rodents and monkeys demonstrates Pb-induced impaired response inhibition by decreased interresponse times and increased overall response rates in FI reinforcement schedules (Table 4-7) (Rossi-George et al., 2011), slower acquisition of a DRL schedule (Rice and Gilbert, 1985), and response perseveration on tests of discrimination reversal learning and FR/waiting for reward (Brockel and Cory-Slechta, 1999b, 1998; Gilbert and Rice, 1987). Coherence is found particularly with evidence in

children for decrements in response inhibition as assessed using the stop signal task. Impulsivity in animals was found with relevant gestational, post-weaning, and lifetime (from gestation or birth) Pb exposures, i.e., producing blood Pb levels 11-29 µg/dL. Pb exposure has not consistently induced attention decrements or distractibility in rats and monkeys (Brockel and Cory-Slechta, 1999a; Gilbert and Rice, 1987). The findings in children and animals for Pb-associated impulsivity are supported by evidence that Pb affects dopaminergic neurons of the frontal cortex and striatum of the brain by altering dopamine release and receptor density. The circuitry in these regions is thought to mediate response inhibition. Attention decrements, impulsivity, and hyperactivity also have been linked with changes in the hippocampus, and evidence describing the effects of Pb on hippocampal functions further supports the mode of action for the effects of Pb on these behaviors.

Conduct Disorders

As described at the beginning of Section 4.3.3, there are two domains of conduct disorder: undersocialized aggressive conduct disorder and socialized aggressive conduct disorder. Although not examined as extensively as cognitive function or other externalizing behaviors, previous and recent prospective studies consistently demonstrate Pb-associated increases in delinquent behavior, aggression, antisocial activities, and destructive behavior in children as rated by parents and teachers (Table 4-12). The available evidence does not point to an effect of Pb exposure in one particular domain of conduct disorders. Supporting evidence also is provided by studies showing associations with criminal offenses in adolescents and young adults as assessed with government records. Most studies examined multiple measures of conduct disorders; however, the consistent pattern of association observed across the ages of blood Pb level and/or behaviors examined increases confidence that the evidence is not unduly biased by an increased probability of finding associations by chance alone. Recent cross-sectional studies found associations between concurrent blood Pb level and ratings of conduct disorders, but several had limitations aside from study design (Nigg et al., 2008; Chen et al., 2007; Chiodo et al., 2007), including limited consideration for potential confounding. A particularly informative cross-sectional study was that finding a 7.2 (95% CI: 1.1, 49) higher odds of parentally-assessed conduct disorder in 2,867 children ages 8-15 years participating in NHANES with concurrent blood Pb levels 0.8-1.0 µg/dL compared with blood Pb levels 0.2-0.7 µg/dL (Braun et al., 2008). There were 22 children rated as having conduct disorder in the group with blood Pb levels 0.8-1.0 µg/dL, which may have contributed to the wide 95% CI. The association also could have been influenced by higher past Pb exposures of the children. Further, while adjustment was made for age, sex, race, poverty to income ratio, and smoking exposure, potential confounding by

parental caregiving quality was not examined. Evidence of Pb-induced aggression in animals was mixed in adult animals with lifetime Pb exposure beginning in gestation and not indicated in juvenile animals.

The evidence from prospective studies for ratings of behaviors related to conduct disorders in children is substantiated by analyses of school-aged children and young adults (ages 7-17 years) in populations from various locations and SES (i.e., U.K., Cincinnati, Australia) with high participation rates, lack of indication of substantial selection bias, and consideration of several potential confounding factors including multiple SES-related factors, parental caregiving quality, smoking exposure, and birth outcomes (Chandramouli et al., 2009; Dietrich et al., 2001; Burns et al., 1999). Pb biomarker levels were associated with both parent and teacher ratings of behaviors related to conduct disorders, reducing the likelihood of higher behavioral ratings biased to parents of children with higher Pb biomarker levels. Recent prospective studies of criminal offenses in adolescents and young adults strengthened previous evidence with consideration for potential confounding by factors such as SES, smoking, drug, and alcohol exposure, and parental caregiving quality (Fergusson et al., 2008; Wright et al., 2008). In the Cincinnati cohort, a 1 µg/dL increase in age 6 year blood Pb level was associated with an increased risk of criminal arrests with an RR of 1.05 (95% CI: 1.01, 1.09) with adjustment for maternal IQ and education, sex, and SES (Wright et al., 2008). In the New Zealand cohort, a log unit increase in tooth Pb (from ages 6-8 years) was associated with a 0.49 (95% CI: 0.16, 0.82) increase in the number of documented property or violent convictions at ages 14-21 years (Fergusson et al., 2008) with adjustment for SES, ethnicity, parental criminal offenses, and parental alcoholism. Further support for Pb-associated increases in conduct disorders was provided by a recent meta-analysis that found that the evidence was robust to heterogeneity in study design, definition and assessment method of conduct disorders, potential confounding factors examined, and population mean blood Pb levels (Marcus et al., 2010). As described in Section 4.3.3, there is not evidence to support parental psychopathology as a direct confounding factor. There hypothetically could be potential confounding by parental psychopathology via a relationship with parental caregiving quality. However, Pb-associated increases in behaviors related to conduct disorders have been found with adjustment for parental caregiving quality.

Associations of measures of conduct disorders at ages 7-24 years (parent/teacher behavior ratings and criminal offenses) with earlier childhood blood (e.g., age 30 month, age 6 year), early childhood average blood (e.g., age 0-6 year), lifetime average blood (to age 11-13 years), tooth, and bone Pb levels pointed to the effects of early childhood or cumulative Pb exposures. Associations were found with a mean lifetime average (to age 11-13 years) blood Pb level of 14 µg/dL, mean age 6 year blood Pb level of 6.8 µg/dL,

mean prenatal maternal blood Pb level of 7.8 µg/dL, and age 30 month blood Pb levels >10 µg/dL. Recent cross-sectional studies found associations with concurrent blood Pb level and lower blood Pb levels, means 1 and 5 µg/dL (ages 7-17 years), but the study limitations detailed above limit strong inferences regarding the effects of Pb exposure on conduct disorders in these populations. Most prospective studies did not analyze Pb biomarker levels at multiple lifestages and time periods, including later childhood and more recent adulthood, or examine differences in association between Pb biomarker levels measured at various lifestages and time periods. The evidence does not identify an individual critical lifestage, time period, or duration of Pb exposure associated with conduct disorders in children or exclude an effect of more recent Pb exposures.

4.3.4 Internalizing Behaviors in Children

4.3.4.1 Epidemiologic Studies of Internalizing Behaviors in Children

Most studies of the effects of Pb on behavior in children have focused on externalizing behaviors such as attention, impulsivity, and conduct disorders. However, several studies also have linked biomarkers of Pb exposure in children with internalizing behaviors characterized by directing feelings and emotions inward, i.e., withdrawn behavior, symptoms of depression, fearfulness, and anxiety. As with externalizing behaviors, internalizing behaviors can be classified into various domains: withdrawn behavior, somatic symptoms, anxiety/depression. Studies examining relationships with Pb exposure examined multiple behaviors and did not clearly indicate that Pb exposure affected a particular domain of internalizing behaviors. Therefore, the evidence for internalizing behaviors is examined as a single group. Some studies found stronger associations for externalizing behaviors than for internalizing behaviors (Plusquellec et al., 2010; Wasserman et al., 2001; Bellinger et al., 1994a; Sciarillo et al., 1992). Others did not find a clear difference in the strength of association (Roy et al., 2009; Chiodo et al., 2004; Bellinger et al., 1994b), which is not unexpected since externalizing and internalizing behaviors are positively correlated. Internalizing behaviors frequently were assessed using the Child Behavior Checklist, and as with externalizing behaviors, were rated by parents and/or teachers. Associations with both parent and teacher ratings increase confidence that biased reporting of internalizing behaviors by parents of children with higher blood Pb levels did not unduly influence the collective body of evidence. Most studies had moderate to high follow-up participation. With the exception of the Yugoslavia cohort, participation was not biased to those with higher ratings of internalizing behaviors and higher blood Pb levels. Additionally, in most studies, a

consistent pattern of association was observed across the ages of blood Pb level and/or multiple behaviors examined, which increases confidence that the evidence is not strongly biased by an increased probability of finding associations by chance alone.

The potential for parental psychopathology to influence associations between child Pb and externalizing behaviors was described in Section 4.3.3. Parental psychopathology has the potential to influence associations between child Pb and internalizing behaviors in the same ways since internalizing behaviors also have a familial component. As with externalizing behaviors, available evidence does not support consideration of parental psychopathology as a direct potential confounder of child Pb-internalizing behavior associations. Instead, the ISA evaluates potential confounding by parental caregiving quality (speculated to mediate a potential correlation between parental psychopathology and Pb exposure) and other factors well documented in the literature to be correlated with both Pb exposure and internalizing behaviors such as SES and parental education.

Key evidence was provided by prospective studies in various populations, i.e., Boston, Port Pirie, Australia, and Yugoslavia. Collectively, these studies found higher ratings for internalizing behaviors in children (n = 322-1,273, ages 3-13 years) in association with cord blood, concurrent blood (age 3 year), lifetime average blood, and tooth Pb levels (Wasserman et al., 2001; Burns et al., 1999; Wasserman et al., 1998; Bellinger et al., 1994b). In the Port Pirie cohort, Burns et al. (1999) found that higher lifetime average blood Pb levels (mean: ~14 µg/dL) were associated with parental ratings of externalizing behaviors more strongly in boys and with internalizing behaviors (i.e., withdrawn, anxious/depressed, composite) more strongly in girls ages 11-13 years, which may indicate sex differences in the effect of Pb or differences in the types of behaviors that are observed and reported in girls versus boys. Based on a log-linear model, a 1 µg/dL increase in lifetime average blood Pb level was associated with increased odds of an anxious/depressed rating above the median of 1.04 (95% CI: 1.0, 1.09) among 159 boys ages 11-13 years (in the 10th-90th percentile interval of blood Pb level 13.7-14.9 µg/dL) and 1.07 (95% CI: 1.01, 1.14) among 163 girls ages 11-13 years (in the 10th-90th percentile interval of blood Pb level 13.3-14.9 µg/dL). These associations were found with the adjustment for SES factors, HOME score, family functioning score, and current maternal psychopathology (General Health Questionnaire).

Differences between externalizing and internalizing behaviors also were found in the Yugoslavia cohort but by age of assessment and blood Pb levels. This cohort was examined between ages 3 and 5 years, ages at which behaviors may be less reliably assessed. Among 379 children ages 3 years from the higher and lower Pb exposure towns, higher cord and concurrent blood Pb levels (respective means: 14, 25.8 µg/dL) were associated with higher maternal ratings of anxious-depressed, withdrawn, and

externalizing behaviors, with stronger associations found for concurrent blood Pb level (Wasserman et al., 1998). Among 191 children ages 4-5 years from the lower Pb exposure town, higher lifetime average blood Pb level (mean: 7.2 µg/dL) was associated with higher ratings of delinquent behavior and internalizing behaviors, with stronger associations found for delinquent behavior (Wasserman et al., 2001). A log increase in higher lifetime average blood Pb level was associated with a 0.22 log higher (95% CI: -0.03, 0.47) rating of withdrawn behavior and 0.19 log higher (95% CI: -0.05, 0.43) rating of anxious-depressed behavior. Results at each age were adjusted for HOME score, sex, ethnicity, and maternal education. Additional covariates included residence type at age 3 years and age, birthweight, and maternal smoking history at ages 4-5 years.

With regard to important lifestages or durations of Pb exposure, results from prospective studies did not clearly demonstrate differences in association among Pb biomarkers measured at various lifestages or time periods. The importance of cumulative Pb exposure was indicated by associations found with lifetime average blood Pb levels in the Port Pirie cohort (to age 11-13 years) and in the Yugoslavia cohort (to age 5 years) and with tooth Pb (from age 6 years) levels in Boston children at age 8 years. In Boston children, Pb levels measured in teeth (mean: 3.4 µg/g) but not cord blood were associated with a teacher rating of internalizing behavior score > 60 at age 8 years (Bellinger et al., 1994b). In another Boston-area cohort, tooth (collected at first or second grade) Pb levels were not associated with self-rated symptoms of depression (Profile of Mood States questionnaire) at ages 19-20 years (Bellinger et al., 1994a). Prospective studies did not analyze a detailed history of Pb biomarker levels to evaluate persistence of effects of early exposure or to identify an individual critical lifestage or time period of Pb exposure associated with increases in internalizing behaviors. The available evidence does not preclude an effect of later childhood or more recent Pb exposure.

In the Cincinnati cohort, Dietrich et al. (1987) with structural equations showed that associations of prenatal maternal and age 10 day blood Pb level (respective means: 8.3, 4.9 µg/dL) with poorer mood in infants ages 6 months (n = 185) were indirect, meaning they were mediated through associations of Pb with shorter gestation or lower birth weight. These results suggest that Pb may exert its effects by impairing nervous system development. The fetal period is an active period of neuronal differentiation, dendritic branching, and synaptogenesis, which if impaired by Pb exposure, could have broad implications on subsequent neurodevelopment. There are few such analyses, and the implications are limited by the lower reliability of mood assessed in infancy.

Cross-sectional studies found associations between concurrent blood (Liu et al., 2011b; Roy et al., 2009) or hair (Bao et al., 2009) Pb levels and teacher and parent ratings of internalizing behaviors in children in China and India (n = 303-756, ages 3-16 years).

Mean concurrent blood Pb levels in children ages 3-7 years were 11 and 14 µg/dL. Results were adjusted for family income and parental education but not caregiving quality. In the few studies of populations with mean blood Pb levels ~5 µg/dL, results were inconsistent. Chiodo et al. (2004) found an association with internalizing behaviors with adjustment for SES, maternal hard drug use, and marital status in 175 children age 7.5 years in Detroit, MI who had high prevalence of prenatal alcohol exposure. HOME score, maternal education, and prenatal alcohol and drug exposure were not found to influence associations with blood Pb level. While prenatal alcohol and drug exposure may not affect the internal validity of results, they may limit generalizability of the results to the general population of U.S. children. A study that examined 79-91 Inuit children (age 5 years) in Quebec, Canada with Hg and PCB co-exposures from a high fish diet, did not find statistically significant associations between concurrent blood Pb level and internalizing behaviors with consideration of potential confounding by parental caregiving quality, caregiver education and IQ, blood Hg or PCB levels, and prenatal smoking and alcohol exposure (Plusquellec et al., 2010).

Associations of Pb biomarkers with internalizing behaviors in children were observed with consideration for a wide range of potential confounding factors, most commonly, age, birth outcomes, parental education, and other SES-related factors. Parental caregiving quality was evaluated in few studies. Blood Pb-associated higher ratings of internalizing behaviors were found with adjustment for HOME score in the Yugoslavia cohort (Wasserman et al., 2001; 1998), and HOME, family functioning, and current maternal psychopathology (General Health Questionnaire) in the Port Pirie cohort (Burns et al., 1999). Several studies, each of which adjusted for a different set of covariates, found slightly attenuated or larger effect estimates in multivariate models than unadjusted models (Wasserman et al., 2001; Burns et al., 1999; Bellinger et al., 1994b). Collectively, these observations increase confidence that the associations observed with Pb biomarkers reflect a relationship with Pb exposure.

4.3.4.2 Toxicological Studies of Internalizing Behaviors

As in epidemiologic studies, toxicological studies have focused more on cognitive function and externalizing behaviors and less on internalizing behaviors. The associations described in the preceding section for Pb biomarker levels with ratings of withdrawn behavior and symptoms of depression and anxiety in children are supported by findings of Pb-induced anxiety, emotionality and depression-like behaviors in animals.

Emotionality has been indicated by increased disruption in performance and frustration in response to errors and reward omission in visual or olfactory discrimination task trials in

Pb-exposed rats (Beaudin et al., 2007; Stangle et al., 2007). In each study, Long-Evans rats were exposed to Pb during and after lactation (300 ppm Pb acetate via dam drinking water PND1-PND17 then either 20 or 300 ppm PND17-PND30 in own water, with respective blood Pb levels of 13 and 31 µg/dL on PND52). However, this exposure regimen in mice was reported to produce higher peak blood Pb levels at the end of exposure, i.e., 40-60 or 100-140 µg/dL. In Beaudin et al. (2007), greater disruption in performance (i.e., failure to enter testing alcove) after committing errors and having rewards omitted was found in rats with blood Pb levels 13 and 31 µg/dL tested at age 9-15 weeks. Pb-exposed rats also had greater response latency with reward omission as indicated by the entrance into the testing alcove but failure to respond within a set period of time. In Stangle et al. (2007), increased reactivity to errors and reward omission was found in rats with blood Pb levels 31 µg/dL. In rhesus monkeys, emotional dysregulation was indicated by tactile defensiveness after exposure to Pb acetate/50% glucose in 4 cc daily milk formula from PND8 to ages 1-2 years to produce target blood Pb levels 35-40 µg/dL (Moore et al., 2008).

In other studies, rats showed emotionality and depression-like behavior in the open field test or forced swim test (i.e., Porsolt's Test) with Pb exposure. The open field test monitors activity levels and movements of animals in three dimensions. Depression-like behavior is indicated by freezing behavior and low levels of activity. Emotionality is indicated by grooming or freezing. In the forced swim test, animals are placed in a vertical cylinder of water from which there is no escape and monitored for duration of struggling or attempt to escape. Animals that stop quickly are ascribed a depression-like phenotype. As for many other neurobehavioral responses, sex-specific differences were found. Pb-exposed male Wistar rats showed increased emotionality in the open field test as indicated by fewer counts of rearing and longer time freezing. Pb-exposed females showed a depression-like phenotype in the forced swim test as indicated by longer time of immobility (de Souza Lisboa et al., 2005). While measured blood Pb levels of rats were low, 5-7 µg/dL, they were measured after a lag in exposure (PND70) and produced by oral gavage (10 mg/day to dams), a route that may be less relevant to human routes of Pb exposure. Pb-induced immobility in the forced swim test also was found with 6-week postnatal (PND30-PND72) Pb acetate exposure via drinking water but producing a mean blood Pb level of 40 µg/dL 6-7 weeks after exposure ceased. Reducing internal Pb dose with the chelator succimer reversed Pb-induced immobility (Stewart et al., 1996).

Depression initially may seem like an unexpected effect of immune modulation, but it has been linked to an interaction between the CNS and the immune system via alterations in cytokines such as IL-6 (Section 4.6.6.1). Dyatlov and Lawrence (2002) found that dietary Pb exposure through lactation and a brief period after weaning (500 µM, PND1-PND22, resultant blood Pb level: 17 µg/dL) potentiated sickness behavior in mice in response to

bacterial infection. Sickness behavior was evidenced by an increase in serum IL-6 levels with an accompanying decrease in food and water intake and decrease in body weight gain. This phenotype was correlated with decreases in the populations of several T cell subtypes. Pb exposure also potentiated release of IL-1β, which plays an important role in inflammatory responses to infection and has been shown to inhibit hippocampal glutamate release in young but not aged animals. Sickness behavior also was induced in Pb-exposed animals with IL-6 and IL-1 administration without infection, further supporting a role for immunomodulation in mediating sickness behavior.

Pb exposure had mixed effects on anxiety-related responses as measured by the elevated plus maze, which assesses behavior of rodents in an unfamiliar environment. The maze is elevated above the floor and consists of two arms that are enclosed with walls intersected with two arms that have no walls. The animal is placed in the center of the maze, and longer latency to enter an open arm, and lower frequency and duration of entries into an open arm are indicative of anxiety. Postnatal (PND1-PND30) exposure of female rats to 2,000 ppm Pb acetate in drinking water, yielding blood Pb levels 34 µg/dL, induced an increase in anxiety-related behavior at PND60, as indicated by a lower percentage of open arm entries and less time spent in the open arms (Fox et al., 2010). In a study of gestational/lactational (GD1-PND24) exposure to 2.84 mg/mL Pb acetate trihydrate in drinking water, Pb-exposed Sprague-Dawley rats did not differ in anxiety-related responses from controls. Blood Pb levels of these Pb-exposed rats were higher than those relevant to this ISA, 69.8 µg/dL at PND25 (Molina et al., 2011).

4.3.4.3 Summary of Internalizing Behaviors

Internalizing behaviors, i.e., withdrawn behavior, symptoms of depression and anxiety, have been examined less than externalizing behaviors (e.g., attention, conduct disorders). However, several prospective studies found associations of higher parental and teacher ratings of internalizing behaviors with higher tooth or lifetime average blood Pb levels in children ages 8-13 years (Burns et al., 1999; Bellinger et al., 1994b) and higher cord, concurrent, and lifetime average blood Pb levels in children ages 3-5 years (Wasserman et al., 2001; 1998). Collectively, the lack of biased participation by subjects with higher blood Pb levels and associations found with both parent and teacher ratings increase confidence that the evidence is not unduly influenced by biased reporting of behaviors by parents of children with higher blood Pb levels. The prospective studies found associations with adjustment for several potential confounding factors, including age, birth outcomes, SES, parental education, and parental caregiving quality. As described in Section 4.3.3, there is not evidence to support consideration of parental psychopathology as a direct confounding factor. There hypothetically could be potential confounding by

parental psychopathology via a relationship with parental caregiving quality. However, Pb–associated increases in internalizing behaviors have been found with adjustment for parental caregiving quality. In prospective studies, associations were found with tooth Pb levels and lifetime average, cord, and concurrent blood Pb levels. While, there is not a clear indication of an individual critical lifestage or time period of Pb exposure associated with internalizing behaviors, several observations point to an effect of cumulative Pb exposure. These results do not preclude an effect of later childhood or more recent Pb exposures. In prospective studies, associations with internalizing behaviors were found with mean lifetime average blood Pb levels of 14 µg/dL (to age 11-13 years) (Burns et al., 1999) and 7.2 µg/dL (to age 4.5-5 years) (Wasserman et al., 2001). In the Yugoslavia cohort at ages 4-5 years, in whom behaviors may be less reliably rated, lifetime average blood Pb level was associated more strongly with the rating of delinquent behavior than ratings of internalizing behaviors (Wasserman et al., 2001). Cross-sectional studies found concurrent blood Pb-associated increases in internalizing behaviors in children ages 3-16 years. Studies that considered potential confounding by a similar set of factors as the prospective studies examined children ages 5-7 years with mean concurrent blood Pb levels ~5 µg/dL and found inconsistent associations (Plusquellec et al., 2010; Chiodo et al., 2004).

Evidence in children is supported by observations that dietary Pb exposure resulted in depression-like behavior and emotionality in rodents (gestational-lactational, PND1 to PND 22 or 30, post-lactation) and rhesus monkeys (neonatal exposure to age 1-2 years) (Moore et al., 2008), with some evidence in rodents at blood Pb levels relevant to this ISA (13, 17 µg/dL) (Beaudin et al., 2007; Dyatlov and Lawrence, 2002). Evidence for Pb-induced anxiety in animals is mixed. Mode of action support is provided by well-documented evidence for Pb-induced changes in the HPA axis (Section 4.3.2.3) and dopaminergic and GABAergic systems (Sections 4.2.2.2, 4.3.10.4, and 4.3.10.8), which are involved in regulating mood and emotional state.

4.3.5 Psychopathological Effects in Adults

4.3.5.1 Epidemiologic Studies of Psychopathological Effects in Adults

The potential effects of Pb exposure on psychopathological effects (e.g., anxiety, depression, schizophrenia) have been examined less so in adults than in children and less than cognitive function in adults. Most studies of Pb and psychopathologies in adults have examined mood states, which are an integral part of the neurocognitive test battery

of the World Health Organization (WHO). There is evidence that indices of the Profile of Mood States may be particularly sensitive to toxicant exposures (Johnson et al., 1987). As with other nervous system endpoints in adults, evidence reviewed in the 2006 Pb AQCD (U.S. EPA, 2006b) pointed to effects in Pb-exposed workers. Higher prevalence of self-reported symptoms of depression, anxiety, and tension was found among Pb-exposed workers (n = 43-576, mean concurrent or peak blood Pb levels: 31-79 µg/dL) in association with higher blood Pb levels or compared with unexposed controls (n = 24-181, mean blood Pb levels: 15-38 µg/dL) (Schwartz et al., 2005; Maizlish et al., 1995; Parkinson et al., 1986; Baker et al., 1985; Baker et al., 1984; Lilis et al., 1977). Several studies considered potential confounding by age, sex, and education. Medical conditions, smoking, and alcohol use were examined in one or two studies. Only Maizlish et al. (1995) examined other occupational exposures (previous occupational exposure to solvents). Occupational Mn exposure has been linked with anxiety and depression but was not examined as a potential confounder of Pb associations. Most studies were cross-sectional, which makes less certain the temporal sequence between Pb exposure and development of psychopathological effects.

The few studies of adults without occupational Pb exposures participating in NAS and NHANES demonstrated associations of concurrent blood and bone Pb level with symptoms of depression and anxiety. As bone Pb is a major contributor to blood Pb levels in adults without current occupational Pb exposure, cross-sectional associations for both blood and bone Pb may indicate effects of cumulative Pb exposure. Although specific covariates varied among studies, these previous and recent cross-sectional studies found associations with adjustment for several potential confounding factors, including age, education, smoking, alcohol use, employment status, and SES. Further, the examination of multiple exposures and outcomes in these studies reduces the likelihood of participation conditional specifically on Pb exposure and psychopathological effects.

Analyses of 526 men ages 48-70 years in the NAS indicated associations of both higher concurrent blood (mean: 6.3 [SD: 4.2] µg/dL), patella (mean: 32.1 [SD: 19.8] µg/g), and tibia (mean: 21.9 [SD: 13.5] µg/g) Pb levels with higher prevalence of a combined index of self-reported depression and anxiety (Rhodes et al., 2003). In a recent analysis of 744 NAS men ages 48-94 years, Rajan et al. (2007) found associations of symptoms assessed using the Brief Symptom Inventory with patella and tibia Pb levels (measured within 1.3 years of outcomes). A 14 µg/g increase in tibia Pb level was associated with an increased odds of an anxiety score one standard deviation above the mean of 1.18 (95% CI: 0.98, 1.42) and of depression score above the median of 1.16 (95% CI: 0.97, 1.38). Similar effect estimates were found for patella Pb level. Effect modification by ALAD genotype was not in a consistent direction. For most of the evaluated mood symptoms, ORs for tibia bone Pb levels were larger among the 587 men with the ALAD 1-1 genotype. In

contrast, ORs for associations between patella Pb levels and symptoms such as depression and positive symptom distress index were larger among the 119 ALAD-2 carriers. In the NAS, effect modification by ALAD genotype also was inconsistent for associations between tibia Pb levels and cognitive performance (Rajan et al., 2008) (Section 4.3.2.7). The relationship between ALAD-2 genotype and Pb bioavailability is not clear (Section 4.2.3.3).

A recent analysis of 1,987 adults ages 20-39 years participating in NHANES 1999-2004 was the largest study of psychopathological effects in adults and included both men and women of multiple races and ethnicities (Bouchard et al., 2009). However, only concurrent blood Pb levels were available for analysis. Major depressive disorder, panic disorder, and generalized anxiety disorder were examined using the WHO Composite International Diagnostic Interview, which follows DSM criteria. Adults with concurrent blood Pb levels >0.7 µg/dL (detection limit = 0.2, 0.3 µg/dL) had higher prevalence of all three self-reported disorders. Adults in the highest quintile of concurrent blood Pb level (≥ 2.11 µg/dL) had the highest OR for major depressive disorder (OR: 2.3 [95% CI: 1.1, 4.8]) and panic disorder (OR: 4.9 [95% CI: 1.3, 18]) compared with nonsmoking adults with blood Pb levels 0.3-0.7 µg/dL with adjustment for age, sex, race, education, and poverty to income ratio. A monotonic increase in ORs was not found across the quintiles of blood Pb levels. For all endpoints, ORs were larger in analyses excluding current smokers. While associations were found with relatively low concurrent blood Pb levels, there is uncertainty regarding the magnitude, timing, frequency, and duration of Pb exposure that contributed to the observed associations.

In analyses of small cohorts in California and New England born 1959-1966, Opler et al. (2008; 2004) reported associations between higher levels of cord plasma δ-ALA and subsequent diagnosis of schizophrenia spectrum disorder (ascertained from records and structured interview) in adolescents and adults. Because of the lack of direct measurements of Pb biomarker levels, post hoc analysis, and limited consideration for potential confounding, conclusions regarding a relationship between schizophrenia and Pb and are not warranted. Investigators measured δ-ALA levels in stored serum samples as surrogates for Pb exposure only citing previous observations of a high agreement (90%) between samples with δ-ALA levels ≥ 9.05 ng/mL and blood Pb levels ≥ 15 µg/dL. In the California cohort, δ-ALA level ≥ 9.05 ng/mL was associated with schizophrenia spectrum disorder with an OR of 2.43 (95% CI: 0.99, 5.96), adjusted for maternal age at delivery. In pooled analyses of the California and New England cohorts (n = 200), δ-ALA level ≥ 9.05 ng/mL was associated with schizophrenia spectrum disorder with an OR of 1.92 (95% CI: 1.05, 3.52), with adjustment for maternal age and education. An adjusted OR was not presented for the New England cohort alone, and it

appeared that the association in the pooled dataset was influenced more by that found in the California cohort.

4.3.5.2 Toxicological Studies of Mechanisms for Schizophrenia

An environmental origin of schizophrenia was proposed years ago (Tsuang, 2000), and while epidemiologic evidence is inconclusive, toxicological studies have found Pb exposure to induce changes that have been associated with schizophrenia-related effects (Figure 4-10). Pb exposure has been shown to reduce function in the NMDA receptor (NMDAR) and decrease hippocampal neurogenesis. Pb may bind a divalent cation site in the NMDAR and allosterically inhibit glycine binding (Hashemzadeh-Gargari and Guilarte, 1999). Antagonists of the NMDAR glycine site have been shown to exacerbate schizophrenia symptoms in affected individuals and induce a schizophrenic phenotype in unaffected subjects (Coyle and Tsai, 2004). Developmental Pb exposure inhibits neurogenesis in animal models (Section 4.3.10.9). Evidence indirectly suggests that a decrease in hippocampal degenerate gyrus (DG) neurogenesis could be a mode of action for Pb-associated schizophrenia induction. Decreased neurogenesis is seen in patients with schizophrenia (Kempermann et al., 2008; Reif et al., 2006) and animal models of schizophrenia (Maeda et al., 2007), and clozapine, a treatment for schizophrenia, restores hippocampal DG neurogenesis in animal models of schizophrenia (Maeda et al., 2007) (Figure 4-10). These DG pathways are also NMDAR-dependent.

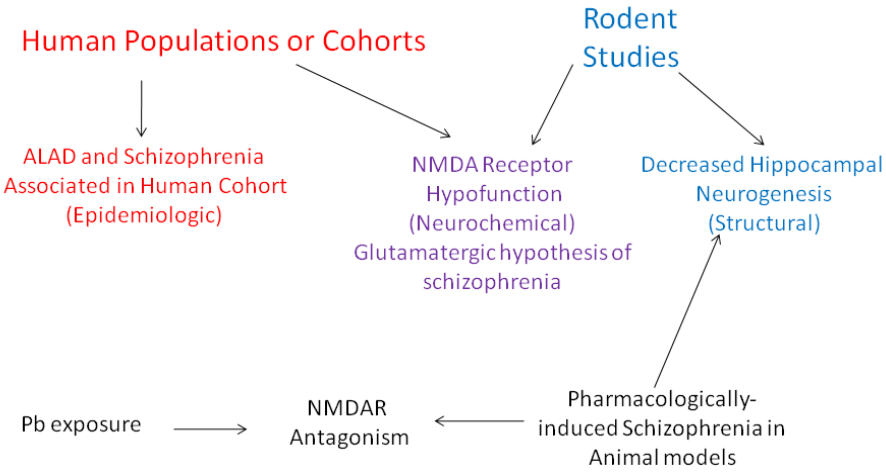


Figure 4-10 Hypothetical representation of the contribution of Pb exposure to the development of a phenotype consistent with schizophrenia.

4.3.5.3 Summary of Psychopathological Effects in Adults

Studies of Pb exposure and behavior in adults have focused on mental disorders rather than the externalizing behaviors that have been examined in children. Evidence links occupational Pb exposure with self-reported increases in symptoms of depression and anxiety, although the cross-sectional design and potential confounding by other occupational exposures such as Mn limit the implications of findings from these occupational studies. However, supporting evidence is provided by a few but large (n = 744 and 1,787) cross-sectional studies in nonoccupationally-exposed adults that found associations of concurrent blood (Bouchard et al., 2009) and bone (Rajan et al., 2007) Pb levels with symptoms of depression and anxiety as assessed with widely-used structured questionnaires such as the Brief Symptom Inventory and the WHO Composite International Diagnostic Interview. Evidence was provided by the NAS study of men (primarily white) and study of men and women (various races/ethnicities) participating in NHANES, both of which involve the examination of multiple exposures and outcomes. Although specific factors varied among studies, studies in adults with and without occupational Pb exposure found associations with adjustment for several confounding factors, including age, sex, and education, and in a few studies, smoking and alcohol use. The cross-sectional nature of these studies makes uncertain the temporal sequence between Pb exposure and development of psychopathological effects and the critical level, timing, frequency, and duration of Pb exposure. Both blood and bone Pb levels in

adults reflect cumulative exposure, and it is uncertain what are the relative contributions of past versus recent Pb exposures to the observed associations. The epidemiologic evidence is supported by observations that Pb exposure (gestational-lactational, lactational, postlactational) induces depression-like and emotional phenotypes in animals (Section 4.3.4.2). The mode of action is supported by evidence for Pb-induced changes in the HPA axis and dopaminergic and GABAergic CNS processes, which are involved in regulating mood and emotional state.

An epidemiologic study found a cord plasma δ-ALA-schizophrenia association but was insufficiently informative because of the use of an indirect biomarker of Pb exposure. However, toxicological studies have shown that Pb exposure decreases NMDAR function and hippocampal DG neurogenesis, which are found in animal models of schizophrenia (agitation, trouble finding food, reduced swimming behavior).

Epidemiologic evidence also indicates associations of Pb biomarker levels with symptoms of depression and anxiety in children and adults as rated by self, parents, or teachers (Section 4.3.4.1). Differences between children and adults for a relationship between Pb and other behaviors may be related to differences in what endpoints are examined. Several studies in children and young adults have examined attention and conduct disorders; studies of older adults did not examine such behaviors. Differential effects in children and adults also may be expected given the predominance of different neurophysiological processes operating at different ages, for example, neurogenesis and brain development in children and neurodegeneration in adults. Differences in the effects of Pb exposure between children and adults also may be related to differences in Pb exposure profiles by age.

4.3.6 Sensory Organ Function

4.3.6.1 Auditory Function

Epidemiologic Studies of Auditory Function in Children

Although not as widely examined as cognitive and behavioral outcomes, several studies found associations of higher blood Pb level with higher hearing thresholds or poorer auditory processing in children, with evidence limited largely to that described in the 2006 Pb AQCD (U.S. EPA, 2006b). The prospective Cincinnati study with repeat measures of blood Pb prenatally to age 5 years provided information on the temporal sequence between Pb exposure and auditory effects and potential critical time periods of

Pb exposure and had extensive consideration for potential confounding. In 215 Cincinnati subjects at age 57 months, poorer auditory processing was associated with higher prenatal maternal, neonatal (age 10-day), yearly age 1 to 5 year (means: 10.6-17.2 µg/dL), and lifetime average blood Pb levels, with the strongest associations found for neonatal blood Pb level (mean: 4.8 [SD: 3.3] µg/dL) (Dietrich et al., 1992). A 1 µg/dL higher neonatal blood Pb level was associated with a 0.26-point ($p \leq 0.10$) and 0.20-point ($p \leq 0.01$) lower score on the total and left ear Filtered Word test (indicating incorrectly identified, filtered, or muffled words), respectively, with adjustment for hearing screen, social class, HOME score, birth weight, gestational age, obstetrical complications, and maternal alcohol consumption. Overall, the findings pointed to a stronger effect of Pb exposure in infancy.

Additional support was provided by the large U.S. cross-sectional NHANES II (n = 4,519) (Schwartz and Otto, 1987) and Hispanic Health and Nutrition Examination Survey (HHANES, n = 3,262) (Schwartz and Otto, 1991) studies. The examination of multiple exposures and outcomes in these studies reduces the likelihood of participation conditional specifically on Pb exposure and hearing function. Each study found an association between higher concurrent blood Pb level and higher hearing thresholds in children (ages 4-19 years). In HHNANES, an increase in concurrent blood Pb level (median: 8 µg/dL) from 6 to 18 µg/dL also was associated with a 15% increase in the percentage of children with a substandard hearing threshold at 2,000 Hz. Higher concurrent blood Pb level also was associated with higher hearing thresholds across several frequencies in a smaller (n = 155) study of similarly aged (4-14 years) children in Poland with similar blood Pb levels (median: 7.2 µg/dL [range: 1.9-28 µg/dL]) (Osman et al., 1999). In each of the studies in children, associations persisted with concurrent blood Pb levels <10 µg/dL. Each of these studies adjusted for different potential confounding factors, but in stepwise regression analyses, most considered parental education, SES, colds, and nutritional factors. Across studies, associations were found with adjustment for factors such as age, sex, ethnicity, family income, concurrent or past colds, antibiotic use, degree of urbanization, and Apgar score (assessment of newborn overall well-being).

Mechanistic support for associations with higher hearing thresholds in children was provided by a few studies that found associations of blood Pb level with lower brainstem auditory evoked potentials in children. Brainstem auditory evoked potentials measure nerve electrical activity and are indicators of neurological auditory function. In prospective analyses of the Mexico City cohort, decreased brainstem auditory evoked potentials were associated with prenatal blood Pb levels at age 9 days (n = 30) (Rothenberg et al., 1994b) and ages 5-7 years and with postnatal blood Pb levels at ages 5-7 years (n = 100-113) (Rothenberg et al., 2000). At age 5-7 years, the shape of the concentration-response relationship differed between prenatal maternal and postnatal

(ages 1 and 4 years) blood Pb level. Linear associations were found for age 1 year and age 4 year blood Pb level (means reported for age 18 and 60 months: 10.8 and 8.0 µg/dL, respectively). Prenatal maternal blood Pb levels showed a biphasic relationship, with lower evoked potentials found with increasing blood Pb levels 1-8 µg/dL and higher evoked potentials found with increasing blood Pb levels 8-30 µg/dL. Results were adjusted for age, sex, and head circumference. Associations with increased latencies of auditory evoked potentials were found in small studies (n = 29, 49) of children and adults with higher blood Pb levels (i.e., range 6-84 µg/dL) than most of the current U.S. population of children (Holdstein et al., 1986; Robinson et al., 1985).

Recent cross-sectional studies aimed to identify the locus in the auditory system affected by Pb exposure in the examination of a population of children (n = 53, 117, ages 2-18 years) living in Pb glazing communities in Ecuador with higher blood Pb levels than those relevant to this ISA (means 34 and 38 µg/dL) (Buchanan et al., 2011; Counter et al., 2011). Concurrent blood Pb level was not correlated with the acoustic stapedius reflex (Counter et al., 2011) or distortion product otoacoustic emissions (Buchanan et al., 2011), indicating lack of effect on the auditory brainstem or inner ear, respectively. Other loci were not examined, and potential confounding was not considered.

Epidemiologic Studies of Auditory Function in Adults

Studies of auditory function reviewed in the 2006 Pb AQCD consistently indicated associations between blood Pb levels and prolonged latencies in auditory evoked brainstem potentials in occupationally-exposed adults (U.S. EPA, 2006b). A few recent studies found increases in hearing thresholds in Pb-exposed workers. A recent analysis of the NAS provided evidence in nonoccupationally-exposed men for associations of tibia Pb levels with hearing loss.

Among 448 men in the NAS, higher tibia Pb level (mean: 22.5 µg/g) at mean age 64.9 years, measured up to 20 years after initial hearing testing, was associated with a faster rate of increase in hearing threshold for frequencies of 1, 2, and 8 kHz and a pure tone average. Men were free of hearing loss at baseline and had hearing tested repeatedly (median 5 times per subject) over a median of 23 years (Park et al., 2010). Blood Pb was not examined. In cross-sectional analyses adjusted for age, race, education, body mass index, pack-years of cigarettes, diabetes, hypertension, occupational noise (based on a job-exposure estimate), and presence of a noise notch (indicative of noise-induced hearing loss), higher patella Pb level (mean 32.5 µg/g, measured within 5 years of hearing test) was associated with a higher hearing threshold for frequencies greater than 1 kHz. A 21 µg/g (interquartile range) increase in patella Pb level was associated with pure tone average hearing loss with an OR of 1.48 (95% CI: 1.14, 1.91) in adjusted analyses.

Similar, but slightly weaker associations were found for tibia bone Pb levels. In the NAS, bone Pb levels were measured after the initial hearing measurement but reverse causation is unlikely since bone Pb is an indicator of cumulative Pb exposure, and tibia Pb has a half-life on the order of decades (Section 3.3). Bone Pb levels increase with age, and although age was included as a model covariate, residual confounding by age is possible.

Recent cross-sectional studies added evidence for associations between higher concurrent blood Pb levels and higher hearing thresholds in adults with potential or actual occupational Pb exposures. A hospital-based case-control study examined workers from diverse occupations and examined potential confounding by other occupational exposures. Cases included workers referred for hearing testing (average hearing thresholds above 25 dB), and controls comprised workers with normal hearing thresholds who were having routine occupational health examinations (Chuang et al., 2007). Geometric mean blood Pb levels were 10.7 μg/dL for the 121 cases and 3.9 μg/dL for the 173 controls. In models that adjusted for age, smoking status, alcohol consumption, years of noise exposure, as well as Mn, arsenic (As), and selenium (Se) levels in blood, higher blood Pb levels were associated with higher hearing threshold (0.5-6 kHz). The potential selection bias arising from the nonrandom population sample may limit implications of these findings. Other studies found associations of higher concurrent blood Pb level with increased hearing thresholds or hearing loss in Pb-exposed workers (n = 183-259) but had limited or no consideration for potential confounding (Hwang et al., 2009; Forst et al., 1997) and/or examined workers with a mean blood Pb level of 57 μg/dL (Wu et al., 2000).

Toxicological Studies of Auditory Function

The 2006 Pb AQCD (U.S. EPA, 2006b) described increases in hearing thresholds or latencies in brainstem auditory evoked potentials in nonhuman primates (n = 5-7) exposed to lifetime Pb from gestation or birth to ages 8-13 years (resulting in blood Pb levels 33-150 μg/dL at peak or during testing) (Rice, 1997; Lilienthal and Winneke, 1996). Rice (1997) found that half of the pure tone detection thresholds were above the control range at certain frequencies. In Lilienthal and Winneke (1996), the Pb-related increased latency in auditory evoked potentials persisted after Pb exposure was terminated, and blood Pb levels had decreased to < 5 μg/dL. The observations in monkeys are consistent with the epidemiologic associations described above but were related to higher Pb exposures than those relevant to this ISA. In addition to indicating hearing loss, brainstem auditory evoked potentials can indicate impaired synaptic maturation and incomplete neuron axon myelination leading to impaired neuronal conduction (Gozdzik-Zolnierkiewicz and Moszyński, 1969). Thus, the findings from Rice (1997) and those described in the preceding sections for children may indicate that Pb

exposure impairs auditory nerve conduction. Studies in animals with blood Pb levels >300 µg/dL found that the cochlear nerve was especially sensitive to Pb exposure (Gozdzik-Zolnierkiewicz and Moszyński, 1969).

A recent study found similar effects in monkeys with similar blood Pb levels as Lilienthal and Winneke (1996), but with only early-life Pb exposure. Laughlin et al. (2009) studied rhesus monkeys exposed to Pb acetate prenatally to birth or postnatally from birth through weaning at age 5.5 months (maternal drinking water, 3 months prior to mating until weaning, resulting in bone Pb levels at age 11 years of 7 and 13 µg/g for prenatal and postnatal groups, respectively, and blood Pb levels averaged over Pb exposure of 35 and 46 µg/dL, respectively). Auditory threshold testing and threshold task testing was conducted at 13 years of age after blood Pb levels had returned to those found in controls. At birth, animals were cross fostered, creating a control group, a prenatal Pb group, and a postnatal Pb group; however, Pb-exposed animals were analyzed as a single group. Pb exposure induced small, statistically nonsignificant elevations in auditory thresholds in animals. Behavioral testing during the auditory threshold task indicated less engagement and responsiveness in Pb-exposed animals. This study has multiple limitations that could have contributed to the lack of statistically significant aberrations, including limited power with the examination of 5 animals per group, the inability of some of the monkeys to engage or focus on the task at hand which resulted in fewer available measurements, differences between the sexes in attention, and mixing of the postnatal Pb and prenatal Pb-exposed animals into one group.

Summary of Auditory Function

Children

Several studies indicated that higher blood Pb levels are associated with decrements in auditory function in children, as evidenced by increases in hearing thresholds. Results from the prospective Cincinnati cohort study (n = 215) provide key evidence for associations of prenatal maternal, neonatal (age 10 day), yearly age 1 to 4 year, and lifetime average blood Pb levels with increased hearing thresholds at age 57 months (Dietrich et al., 1992), and large (n = 3,000-4,000) cross-sectional analyses of children participating in NHANES and HHANES provide supporting evidence for concurrent blood Pb levels (Schwartz and Otto, 1991, 1987). The examination of multiple exposures and outcomes in NHANES and HHANES reduces the likelihood of participation conditional specifically on Pb exposure and hearing function. In the Cincinnati cohort, mean blood Pb levels were 4.8 µg/dL for neonatal and 17.4 µg/dL for lifetime average. In HHANES, the median concurrent blood Pb level was ~8 µg/dL. Across studies, associations were found with adjustment for factors such as age, sex, ethnicity, family

income, concurrent or past colds, antibiotic use, degree of urbanization, and Apgar score. Potential confounding by parental education, nutritional factors, environmental noise, and maternal smoking also was considered.

Mechanistic evidence was provided by observations of associations between blood Pb level with lower auditory evoked potentials in children, particularly associations found in the prospective analysis of children ages 5-7 years in Mexico City with prenatal maternal, age 1 year, and age 4 year blood Pb levels (Rothenberg et al., 2000). Pb-related increases in hearing thresholds and latencies in brainstem auditory evoked potentials also were found in nonhuman primates with lifetime Pb exposure beginning in gestation or birth (Rice, 1997; Lilienthal and Winneke, 1996), although auditory assessments were made in adult animals ages 8-13 years. Shorter-duration Pb exposure from gestation to birth or from birth to age 5.5 months was found to induce small, statistically nonsignificant elevations in auditory thresholds and decreased responsiveness in behavioral testing performed with the threshold task (Laughlin et al., 2009). In animals, auditory effects were found with higher blood Pb levels (i.e., 33-150 µg/dL at peak or testing) than those relevant to this ISA; thus, it is difficult to ascertain biological plausibility for observations in children.

Adults

In adults, increased hearing thresholds and hearing loss were associated with bone Pb levels in 448 NAS men who were unlikely to have had occupational Pb exposures (Park et al., 2010) and with concurrent blood Pb levels in adults with potential occupational Pb exposure. In the NAS, the examination of multiple exposures and outcomes reduces the likelihood of participation conditional specifically on Pb exposure and auditory function. Among NAS men, higher tibia Pb levels were associated with a faster rate of increase in hearing thresholds over a 23 year follow-up with adjustment for age, race, education, body mass index, pack-years of cigarettes, diabetes, hypertension, occupational noise, and presence of a noise notch. Tibia Pb levels were measured up to 20 years after initial hearing testing, and while there is uncertainty regarding the temporal sequence between Pb exposure and changes in hearing thresholds, Pb in tibia has a half-life on the order of decades. Temporality also is difficult to establish in the cross-sectional occupational studies. A hospital-based case-control study found an association between higher concurrent blood Pb levels and higher hearing thresholds among workers with relevant blood Pb levels (means 10.7 and 3.9 µg/dL in workers with and without hearing problems, respectively) (Chuang et al., 2007). Among other factors, results were adjusted for other metal exposures. Similar auditory effects were found in adult animals ages 8-13 years with lifetime Pb exposures but with higher blood Pb levels (i.e., 33-150 µg/dL) than those relevant to this ISA.

4.3.6.2 Visual Function

The sections that follow review studies examining relationships of Pb exposure with visual function as related to effects on the central nervous system. Studies examining other effects on the visual system such as cataracts are described in Section 4.9.5.

Epidemiologic Studies of Visual Function

The little available epidemiologic evidence does not clearly describe an effect of relevant Pb exposures with effects on the retina in humans. Among children (n = 100-113, ages 7-10 years) in Mexico City examined prospectively, Rothenberg et al. (2002b) reported an association between maternal first trimester blood Pb levels 10.5-32.5 µg/dL and supernormal electroretinograms (ERGs), the impact of which on visual impairment is not clear. In a case-control study of macular degeneration (condition due to retinal damage) in adults, Pb concentrations were elevated in retinal tissue of 36 cases (50 eye donors at time of autopsy; median [IQR]: 12.0 [8-18] ng/g Pb) versus 25 controls (72 eyes; median [IQR]: 8.0 [0-11] ng/g Pb) (Erie et al., 2009). The implications of results in children and adults are limited by the lack of rigorous statistical analysis and consideration for potential confounding. The macular degeneration results are limited further by the inability to establish directionality of effects and uncertainty in representativeness of the control population.

Toxicological Studies of Visual Function

As described in the 1986 and 2006 Pb AQCDs (U.S. EPA, 2006b, 1986b), a majority of the evidence for the effects of Pb on the visual system has been provided by toxicological studies. Pb exposure of animals during perinatal development and adulthood has been found to reduce visual acuity and produce changes describing potential mechanisms of action such as alterations in the retina (Fox et al., 1997; Fox and Sillman, 1979), CNS visual processing areas (Costa and Fox, 1983), and subcortical neurons involved in vision (Cline et al., 1996). For example, environmentally-relevant doses of Pb (10^{-3} µM) in tadpoles inhibited the growth of neurons in the subcortical retinotectal pathway, the main efferent from the retina (Cline et al., 1996). Pb-related aberrations in electrical responses in retinal cells, as measured by ERGs, have been found in rodents and nonhuman primates. Recent research on ERGs expands upon the extant evidence by examining animals with lower Pb exposures or blood Pb levels.

Work in nonhuman primates with Pb exposure during development or over a lifetime (peak blood Pb levels 50-115 µg/dL) showed dysfunction in temporal visual function (responses to different frequencies of light flicker) under high luminance but no change

in spatial function (Rice, 1998). A recent study found no effects of infancy Pb exposure (PND8-age 26 weeks via commercial milk formula, producing blood Pb levels of 35-40 μg/dL) on spatial acuity of rhesus monkeys as assessed with the modified Teller preferential looking paradigm (Laughlin et al., 2008). In monkeys, effects on vision were tested with higher Pb exposures than those relevant to this ISA. Low-level developmental Pb exposure was found to result in sensorimotor deficits in adult zebrafish (Rice et al., 2011). Fish that were exposed as embryos (2 to 24 hours post-fertilization) to water containing 0.03 μM $PbCl_2$ had impaired response to visual stimulation (a rotating bar) under low light conditions. These zebrafish also failed to respond normally to mechanosensory stimulation (0.01 and 0.03 μM $PbCl_2$), showing a significantly impaired startle response.

Animal toxicological evidence also shows that the lifestage of exposure and the dose of Pb contribute to the complex and variable effects of Pb on ERGs (summarized in Table 4-13). The biological relevance of these variable findings is uncertain. Female rats exposed postnatally to 200 or 2,000 ppm Pb acetate exposure via dam drinking water from birth through lactation, resulting in blood Pb levels of 19 and 59 μg/dL at weaning, respectively, had subnormal scotopic ERGs (decreased A- and B-wave amplitudes) with decreased sensitivity and temporal resolution when assessed at 90 days of age (Fox et al., 1991). Similar results were obtained in multiple studies conducted in in vitro models (Otto and Fox, 1993; Fox and Farber, 1988; Fox and Chu, 1988). Monkeys exposed to relatively high levels of Pb continuously from the prenatal period to age 7 years (350 or 600 ppm Pb acetate, resulting in blood Pb levels of 40 and 50 μg/dL, respectively) had persistently increased maximal ERG amplitude (B-wave only, supernormality) and increased mean ERG latency when assessed 2 years after Pb exposure was terminated when blood Pb levels were <10 μg/dL (Lilienthal et al., 1988).

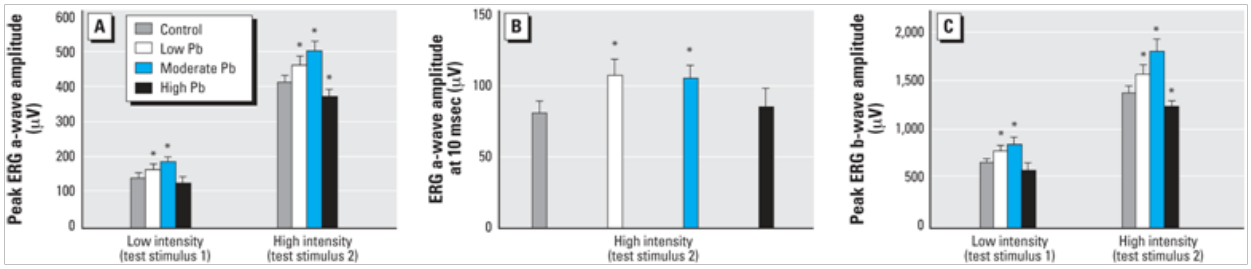
Table 4-13 Summary of Pb-related effects observed on the visual system.

Study, Species, Sex	Pb Exposure Protocol and Dose	Maximal Blood Pb Level (µg/dL)	ERG Effect	Progenitor Cell Pro-liferation	Retinal Cell Apoptosis	Retinal Dopamine Levels	Retinal Cell Layer Thickness
Fox et al. (2008) Long-Evans Rat, F	DW, Prenatal-PND10						
	Low, 27 ppm	12	Supernormal	Yes	Not affected	Dose-dependent ↓	↑
	Moderate, 55 ppm	24	Supernormal	Yes	Not affected	Dose-dependent ↓	↑
	High, 109 ppm	46	Subnormal	No	Yes	Dose-dependent ↓	↓
Lilienthal et al. (1988) Rhesus Monkey, M & F	DW, Prenatal-lifetime						
	350 ppm	~50	Supernormal	—	—	—	—
	600 ppm	~115	Supernormal	—	—	—	—
Fox et al. (1997) Long-Evans Rat, F	DW, PND1-21						
	200 ppm	19	Subnormal	—	Yes	—	↓
	2,000 ppm	59	Subnormal	—	Yes	—	↓
Rothenberg et al. (2002b) Human children, M & F	Prenatal maternal 1st trimester blood	10.5-32.5	Supernormal	—	—	—	—

F = females, M = males, DW = Drinking water exposure, - denotes not examined in the study.

Pb exposure beginning in the gestational period (Pb acetate in drinking water from 2 weeks before mating to PND10) also induced supernormal ERGs in adult Long-Evans rats, with low (27 ppm) and moderate (55 ppm) doses that produced blood Pb levels 10-12 µg/dL and 21-24 µg/dL (Fox et al., 2008) (Figure 4-11 and Table 4-13). This exposure window represents the developmental period for the retina of the rat and is analogous to gestational human retinal development. Subnormal ERGs were induced by the high 109 ppm dose (Figure 4-11), which produced blood Pb levels 40-46 µg/dL. Results of this rodent study demonstrated persistent supernormal scotopic rod photoreceptor-mediated ERGs in animals with blood Pb levels relevant to this ISA. These findings were consistent with the association observed between supernormal ERGs and prenatal maternal blood Pb levels 10.5-32.5 µg/dL in male and female children (Rothenberg et al., 2002b). The functional relevance of findings is uncertain as

supernormal scotopic ERGs may be recorded without other overt ophthalmologic changes and are rarely seen in the clinical setting (Terziivanov et al., 1982).



Note: *p <0.05. Low Pb = 27 ppm Pb acetate, blood Pb level 10-12 µg/dL, Moderate Pb = 55 ppm, blood Pb level 21-24 µg/dL, High Pb = 109 ppm, blood Pb level 40-46 µg/dL. Relative to controls (gray bars), low (white bars) or moderate (blue bars) Pb exposure from gestation through postnatal day (PND)10 induced supernormal ERGs whereas high Pb exposure (black bars) induced subnormal ERGs.

Source: Fox et al. (2008)

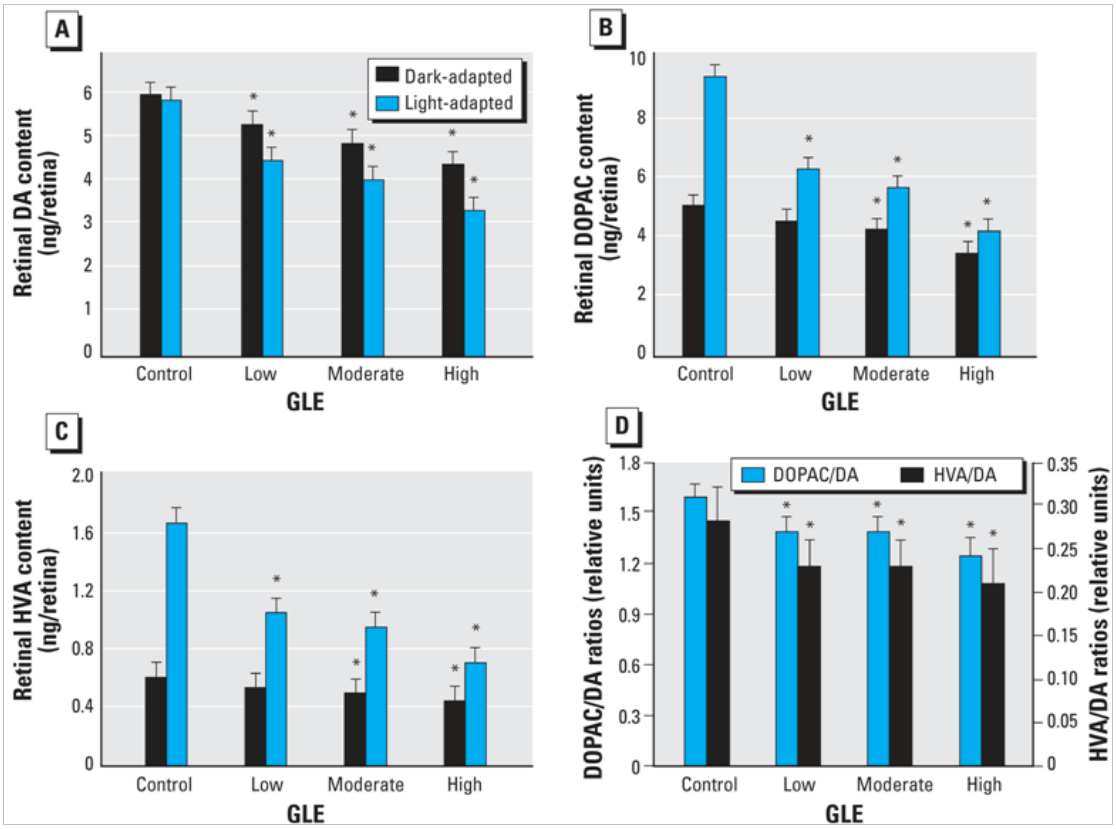
Figure 4-11 Retinal a-wave and b-wave electroretinogram (ERG) amplitude in adult rats after prenatal plus early postnatal Pb exposure.

Animal studies indicate that the dose of Pb and the exposure lifestage not only differentially affect functional tests, i.e., ERG, but also differentially affect retinal cell numbers and morphology. Concomitant with Pb-induced supernormal ERGs, Fox et al. (2008) found that 27 and 55 ppm gestational plus early postnatal Pb exposure increased neurogenesis of rod photoreceptors and rod bipolar cells without affecting apoptosis of Müller glial cells and increased the number of rods in central and peripheral retina (Table 4-13). Concomitant with subnormal ERGs, higher-level gestational plus early postnatal Pb exposure (109 ppm, blood Pb level 40-46 µg/dL) decreased the number of rods in the central and peripheral retina (Fox et al., 2008). Early postnatal (PND1-PND21) Pb exposure (200 or 2,000 ppm, producing blood Pb levels 19 and 59 µg/dL) induced scotopic ERG subnormality in adult rats, decreased the number of rods in the central and peripheral retina, and decreased the retinal Zn concentration (Fox et al., 1997) (Table 4-13). Similar observations were made in separate work in mice. Low and moderate doses of Pb from gestation to PND10 (27 or 55 ppm Pb acetate in dam drinking water, producing blood Pb levels of 12 and 25 µg/dL, respectively) induced greater and prolonged rod bipolar cell neurogenesis and greater thickness and cell number of the outer and inner neuroblastic layers of the retina (Giddabasappa et al., 2011). As in rats, with a higher dose of Pb (109 ppm Pb acetate, resulting in blood Pb levels of 56 µg/dL), there was no increased rod neurogenesis in mice. Nitric oxide has been shown to regulate retinal progenitor cell proliferation in chick embryos (Magalhaes et al., 2006). Thus,

these authors postulated that impaired NO production may contribute to aberrant retinal cell proliferation (Giddabasappa et al., 2011).

Mechanistic understanding of the effect of Pb on the visual system includes the capability of Pb to displace divalent cations, inhibit physiological enzymes, alter cell proliferation and apoptosis, perturb normal neuroanatomical formation (cytoarchitecture in the brain), and affect neurotransmitters. The effects of Pb on the retina were shown to be mediated by inhibition of cGMP phosphodiesterase (PDE) by Pb (Srivastava et al., 1995; Fox and Farber, 1988). Without regard to Pb exposure, pharmacological inhibition of cGMP PDE has been linked with visual problems including alterations in scotopic ERGs (Laties and Zrenner, 2002). Postnatal Pb exposure of animals (peak blood Pb levels: 19, 59 µg/dL) or in vitro Pb exposure of rods isolated from these animals elevated cGMP which contributed to elevated rod calcium concentration (Fox and Katz, 1992) and subsequently induced apoptotic cell death in a concentration-dependent manner.

Pb has been shown to affect a plethora of neurotransmitters in the brain, and it has recently been shown to affect neurotransmitters in the retina. With the aforementioned gestational to PND10 exposure, Pb induced a concentration-dependent decrease in adult rat retinal synthesis of dopamine, which has functions in retinal growth and development and signal transduction in rods and cones (Fox et al., 2008) (Figure 4-12). As discussed in the 2006 Pb AQCD (U.S. EPA, 2006b), other mode of action support for the effects of Pb on the visual system is provided by observations of Pb-induced decreased Na^+/K^+ATPase activity which have been reported in vitro and in vivo. Also, structural changes from chronic Pb exposure (birth to age 6 years) of monkeys included cytoarchitectural changes in visual projection areas of the brain. Maximum blood Pb levels in the low and high dose groups reached 20 µg/dL and 220 µg/dL, respectively (Reuhl et al., 1989).



Note: *p <0.05. GLE = Gestational Pb exposure to postnatal day 10. Low Pb = 27 ppm Pb acetate, blood Pb levels 10-12 µg/dL, Moderate Pb = 52 ppm, blood Pb levels 21-24 µg/dL, High Pb = 109 ppm, blood Pb levels 40-46 µg/dL. A. DA = dopamine, B. DOPAC = dopamine metabolite, C. HVA = dopamine metabolite, D. DOPAC/DA and HVA/DA = ratio of dopamine metabolite to dopamine. Pb exposure decreased dopamine, DOPAC, and HVA in a concentration-dependent manner in light-adapted animals (blue bars). In dark adapted animals (black bars), Pb exposure decreased dopamine, DOPAC, and HVA but not always in concentration-dependent manner. Pb exposure decreased DOPAC/DA and HVA/DA.

Source: Fox et al. (2008)

Figure 4-12 Retinal dopamine metabolism in adult control and gestationally Pb-exposed (GLE) rats.

Summary of Visual Function

Increasing maternal first trimester blood Pb levels (10.5-32 µg/dL) were associated with supernormal ERGs in children in Mexico City at ages 5-7 years (Rothenberg et al., 2002b). The animal evidence showed ERGs in different directions depending on lifestage of Pb exposure and blood Pb level. Supernormal ERGs were found in adult rats with prenatal plus early postnatal (PND10) Pb exposure that produced blood Pb levels of 12 and 24 µg/dL (Fox et al., 2008). The implication of supernormal ERGs on visual impairment is not clear, and the biological relevance of the nonlinear concentration-response is not clear. In these animals, Pb exposure also increased rod cell neurogenesis

and decreased dopamine. Toxicological studies also found other retinal effects including alterations in morphology and cell architecture, signaling, enzyme inhibition, neurotransmitter levels, neuroanatomical development, cell proliferation, and retinal cell apoptosis. Adult monkeys were found to have supernormal ERGs and decreased temporal visual function with developmental or lifetime Pb exposure (Rice, 1998), but infant monkeys had no change in visual acuity with infancy Pb exposure (Laughlin et al., 2008). In each case, Pb exposures were higher (blood Pb levels ≥ 35 µg/dL) than those relevant to this ISA. Pb-associated visual system effects in human adults are not well characterized and are limited to a case-control study finding higher Pb concentrations in retinal tissue from macular degeneration cases (Erie et al., 2009) that did not perform rigorous statistical analysis or examine potential confounding factors.

4.3.7 Motor Function

Some studies in children have assessed fine motor function, i.e., response speed, dexterity, as part of a battery of neurodevelopmental testing, and most have found associations with blood Pb level. Fewer studies have examined gross motor function, i.e., postural balance, action tremor, agility, but also have found associations with blood Pb level in children. Poorer motor function also was found in Pb-exposed workers.

Key evidence from prospective analyses of the Cincinnati and Yugoslavia cohorts demonstrated associations of blood Pb levels with poorer motor function with either adjustment for or consideration for several potential confounding factors related to SES, parental caregiving quality and education, smoking exposure, birth outcomes, sex, and child health. In the Cincinnati cohort, higher earlier childhood blood Pb levels (age 0-5 year average [median: 11.7 µg/dL] or age 78 month) were associated with poorer fine (n = 195) (Ris et al., 2004) and gross motor function (n = 91) (Bhattacharya et al., 2006) assessed in adolescence (ages 12, 15-17 years). In this cohort recruited from birth, follow-up participation was high and not conditional on blood Pb levels. The fine motor function results were adjusted for maternal IQ, SES, HOME score, sex, and adolescent marijuana consumption. Collectively, these findings suggest the persistence of effects of earlier childhood Pb exposure to later childhood; however, later childhood or concurrent blood Pb levels were not examined. Assessments in Cincinnati cohort children at age 6 years indicated associations of concurrent (mean: 10.1 µg/dL), lifetime average (mean: 12.3 µg/dL), and neonatal (mean: 4.8 µg/dL) but not prenatal maternal (mean: 8.4 µg/dL) blood Pb levels with poorer upper limb dexterity, fine motor composite score (n = 245) (Dietrich et al., 1993a), and poorer postural balance (n = 162) (Bhattacharya et al., 1995). These results were adjusted for HOME score and race. Additional covariates included maternal IQ, SES, and sex for fine motor functions (Dietrich et al., 1993a) and height,

BMI, birth weight, bilateral ear infection, nutritional factors, and foot area for postural balance (Bhattacharya et al., 1995). Blood Pb levels were associated with fine and gross motor function in unadjusted and adjusted analyses, increasing confidence that confounding by the examined covariates did not unduly bias the observed associations. Prospective analysis of the Yugoslavian cohort indicated an association of lifetime average blood Pb level (exact levels not reported) with decrements in fine but not gross motor function at age 4.5 years in 283 children (Wasserman et al., 2000a). Although only 50% of the cohort was examined and participation was greater among children with lower SES and HOME score, participation was not conditional on higher blood Pb levels.

Evidence from cross-sectional studies for associations between motor function and concurrent blood Pb level was mixed, with studies examining mean concurrent blood Pb levels 2-3 µg/dL not finding decrements in motor function. Surkan et al. (2007) found that higher concurrent blood Pb level was associated with better fine motor function as indicated by faster finger tapping speed among 534 children (ages 6-10 years) in New England. Results were adjusted for several potential confounding factors such as age, sex, caregiver IQ, SES, and race. Min et al. (2007) found weak, imprecise associations in 61 similarly aged children (7-16 years) in Korea. Concurrent blood Pb level (mean: 5.0 µg/dL) was associated with greater sway oscillation, action tremor, and alternating arm movements in 110 Inuit children (ages 4-6 years) in Quebec, Canada (Despres et al., 2005) with consideration for potential confounding by factors such as HOME score, maternal education and several nutrient levels. The population of Inuit children was selected from subsistence fishing communities, who also have higher exposure to Hg and PCBs. Several indices of fine and gross motor function were associated with blood Pb level, with adjustment for these other exposures. Poorer fine motor function also was associated with higher concurrent blood Pb level in 814 children ages 3-7 years in India with mean concurrent blood Pb level 11.4 µg/dL with adjustment for similar factors as the aforementioned studies (Palaniappan et al., 2011).

An association of Pb exposure with poorer motor function in adults was found in Pb-exposed workers (Iwata et al., 2005), although implications are limited by the cross-sectional design, high concurrent blood Pb levels (mean: 40 µg/dL), and lack of consideration for potential confounding by other occupational exposures. Among 121 Pb smelter workers in Japan, higher blood Pb level was associated with greater sagittal sway with eyes open ($p < 0.05$) and eyes closed ($p < 0.01$) and transversal sway with eyes closed ($p < 0.05$) with adjustment for age, height, smoking status, and drinking status. A mean benchmark dose level (Budtz-Jorgensen et al., 2001; NRC, 2000) of 14.3 µg/dL was calculated for postural sway from a linear model. A supralinear concentration-response function fit the data slightly better than a linear function did.

Pb exposure has shown mixed effects on endurance, balance, and coordination in animals as measured by rotarod performance, treadmill testing, and visual discrimination task. Lower concentration gestational plus early postnatal (to PND10) Pb exposure (27 ppm, producing peak blood Pb level ≤ 10 µg/dL at PND0-PND10) resulted in significantly poorer rotarod performance (i.e., falling off more quickly) than did higher exposure (109 ppm, blood Pb level: 33-42 µg/dL) in male (but not female) adult mice, indicative of a nonlinear concentration-response relationship (Leasure et al., 2008). Other rotarod experiments examining various speeds of rotarod rotation, higher than relevant Pb exposures (blood Pb levels >59 µg/dL), and/or routes of exposure with uncertain relevance to humans, yielded mixed results (Kishi et al., 1983; Grant et al., 1980; Overmann, 1977). An effect on motor function was indicated by increased alcove latency of rats performing a visual discrimination task but with Pb exposure (PND1-30) blood Pb levels of 31 µg/dL (Stangle et al., 2007). Herring gull chicks injected with a single i.p. bolus dose of Pb (100 mg/kg Pb acetate, selected to represent exposure in the wild) had slower development of motor skills versus control birds, as assessed by the treadmill test (Burger and Gochfeld, 2005).

In summary, epidemiologic evidence demonstrates associations of higher blood Pb levels with poorer fine and gross motor function in children ages 3-17 years. Little evidence is available in adults. Prospective analyses of the Cincinnati and Yugoslavia cohorts (n = 91-283) that considered several potential confounding factors such as SES and child health found associations with earlier childhood blood Pb levels (i.e., age 78 month, 0-5 year average) in adolescents (Bhattacharya et al., 2006; Ris et al., 2004) and neonatal, lifetime average, and concurrent blood Pb levels in children ages 4-6 years (Wasserman et al., 2000b; Bhattacharya et al., 1995; Dietrich et al., 1993a). In the Cincinnati cohort, neonatal blood Pb levels were lower than concurrent or lifetime average blood Pb levels at age 6 years (means 4.8, 10.1, 12 µg/dL, respectively). In cross-sectional studies that examined similar potential confounding factors, results were inconsistent in populations with mean blood Pb levels 2-5 µg/dL (Min et al., 2007; Surkan et al., 2007; Despres et al., 2005). While Pb exposure had mixed effects on motor function in animals, mice with relevant blood Pb levels (peak ≤ 10 µg/dL) after gestational-early postnatal Pb exposure showed poorer balance (Leasure et al., 2008).

4.3.8 Seizures in Animals

Previous studies did not consistently show that Pb exposure induced seizures in animals. Pb-induced seizures were found in male Wistar rats exposed to Pb acetate postnatally (250-1,000 ppm in drinking water PND60-PND90, resulting in blood Pb levels of 20-43 µg/dL), as indicated by a decrease in the elapsed time required to develop the first

myoclonic jerk and tonic-clonic seizure (Arrieta et al., 2005). Also, the dose of the seizure-inducing agent pentylenetetrazol (PTZ) required to induce seizures significantly decreased in all Pb dose groups. Other studies showed no effect of Pb exposure on seizures (Schwark et al., 1985; Alfano and Petit, 1981). In a study of early postnatal Pb acetate exposure (2,000 ppm in drinking water PND1-PND25), Pb had variable effects on induction of seizures in Sprague Dawley rats examined at PND25 or PND50, depending on the convulsant-inducing agent administered (Chen and Chan, 2002). These variable effects were hypothesized to be due to the selective effects on inhibitory and excitatory neurotransmission by age and blood Pb level, which were 47 µg/dL and 11 µg/dL at PND25 and PND50, respectively.

Recent investigation expanded on the work by Arrieta et al. (2005) by showing that Pb exposure may induce seizure activity in another rodent species, BALB/c mice. Adult (ages 2-3 months) male BALB/c mice were exposed to Pb acetate for 30 days via drinking water (range of blood Pb levels 50-400 ppm Pb groups: 6.4-18 µg/dL) (Mesdaghinia et al., 2010). Except for 50 ppm Pb exposure, all other Pb concentrations significantly reduced the thresholds of face and forelimb clonus, myoclonic twitch, running and bouncing clonus, and tonic hindlimb extension. High-level Pb administered by bolus injection (200 mg/kg Pb acetate or 50 mg/kg Pb nitrate, single injection, 2 days, blood Pb levels >90 µg/dL) also induced epileptic form activity or seizures in adult male Wistar rats (Krishnamoorthy et al., 1993).

4.3.9 Neurodegenerative Diseases

4.3.9.1 Alzheimer's Disease

Lower scores on the MMSE, which is widely used as a screening tool for dementia, have been associated with higher bone Pb level in NAS men (Wang et al., 2007a; Weisskopf et al., 2004; Wright et al., 2003) but inconsistently with blood Pb level in adults (Weuve et al., 2006; Nordberg et al., 2000). Direct evidence regarding the effects of Pb exposure on Alzheimer's disease is limited to studies reviewed in the 2006 Pb AQCD (U.S. EPA, 2006b), which did not find higher occupational exposure to Pb (Graves et al., 1991) or Pb level in the brains (Haraguchi et al., 2001) in Alzheimer's disease cases than unaffected controls. Overall, the latter studies have sufficient limitations (e.g., case-control design that may be subject to reverse causation, limited consideration for potential confounding) such that evidence is inconclusive regarding the effect of Pb exposure on Alzheimer's disease.

Despite inconclusive epidemiologic evidence, toxicological evidence indicates that Pb exposure in early life promotes Alzheimer's disease-like pathologies in the brains of aged adult animals. Alzheimer's disease is characterized by amyloid-beta peptide (Ab) plaques, hyper-phosphorylation of the tau protein, neuronal death and synaptic loss. In the last decade, the developmental origins of adult health and disease paradigm and the similar Barker hypothesis have indicated that early life exposures can produce aberrant effects in adults. Bolin et al. (2006) connected oxidative DNA damage in adult rats with indicators of plaque formation after lactational Pb exposure that produced blood Pb levels of 46 µg/dL. Wu and colleagues (2008a) made similar observations with infantile Pb exposure of monkeys. These results suggest the need to directly examine the long-term effects of developmental exposure to toxicants rather than relying on adult exposure alone to predict potential health risks in adults (Dietert and Piepenbrink, 2006).

The fetal basis of amyloidogenesis has been examined extensively by the Zawia laboratory in both rodents and nonhuman primates. Mechanistically, Ab plaques originate from the cleavage of the amyloid precursor protein (APP). In male rats exposed to Pb as infants (200 ppm Pb acetate PND1-PND20 in dam drinking water, resulting in pup PND20 blood Pb level of 46 µg/dL and cortex 0.41 µg/g wet weight of tissue) or as adults, infancy Pb exposure induced APP gene expression in the aged animal brains (Basha et al., 2005). A bimodal response was observed, with a significant increase in APP expression above that in control animals first manifesting in infancy and again in old age (20 months). A concomitant bimodal response was observed in specificity protein 1 (Sp1), a transcription factor involved in gene expression in the early development of an organism and known to be related to APP expression. Ab was also significantly elevated in the aged animals developmentally exposed to Pb. Adult-only (age 18-20 months) exposure to Pb did not alter APP or Sp1 expression or Ab production.

Consistent with findings in rodents, Wu et al. (2008a) found that Pb exposure in infancy (PND1-PND400, 1.5 mg/kg per day in infant formula) resulted in significantly higher gene expression of APP and Sp1 and significantly higher protein expression of APP and Ab in aged female monkey cortex tissue (23 year-old *Macaca fascicularis*) from a cohort of animals established in the 1980s by Rice (1992a, 1990). At PND400, the monkeys had blood Pb levels of 19-26 µg/dL. In old age when amyloid plaques had manifested, blood Pb levels and brain cortex Pb levels had returned to control levels. Together, the rodent and nonhuman primate evidence concurs, and indicates that developmental Pb exposure and not adult-only exposure induces elevations in neuronal Alzheimer's Disease-related plaque proteins in aged animals.

Mechanistic understanding of Ab production and elimination after Pb exposure was examined in human SH-SY5Y neuroblastoma cells exposed to Pb concentrations of 0, 5,

10, 20, and 50 µM for 48 hours. Pb was found to affect two separate pathways to increase Ab. Pb exposure induced both the overexpression of APP and repression of neprilysin, a rate-limiting enzyme involved in Ab metabolism or removal (Huang et al., 2011a). Further mechanistic understanding of how Ab peptide formation is affected by Pb exposure was provided by Behl et al. (2009). The choroid plexus is capable of removing beta-amyloid peptides from the brain extracellular matrix. Pb was shown to impair this function, possibly by inhibiting the metalloendopeptidase, insulin-degrading enzyme, which metabolizes Ab (Behl et al., 2009). In another study, lactational Pb exposure of Long-Evans hooded rat pups induced perturbations in DNA binding of SP1 via its Zn finger protein motif (Basha et al., 2003). This effect of Pb was ameliorated by exogenous Zn supplementation.

An additional study with gestational plus lactational Pb exposure (1,000-10,000 ppm, dam drinking water, resultant offspring blood Pb levels: 40-100 µg/dL) showed that the rodent hippocampus as early as PND21 contained neurofibrillary changes, commonly used a marker for Alzheimer's disease. These changes manifested as hyper-phosphorylated Tau, which comprises neurofibrillary tangles, and increased tau and beta amyloid hippocampal protein levels (Li et al., 2010b). Neurofibrillary changes were accompanied by poorer performance in tests of learning and memory.

In summary, recent studies showed that Pb exposure of rats and monkeys during infancy or during gestation/lactation induced significant increases in neuronal plaque-associated proteins such as Ab-peptide, activation of Ab-supporting transcription factors, and hyperphosphorylation of tau, all of which are pathologies found in humans with Alzheimer’s disease. These pathologies were not found with adult-only Pb exposure of animals, further demonstrating that early life Pb is a critical window for Pb-induced Alzheimer’s-like pathologies in animals. The few epidemiologic studies have not linked higher Pb exposure with Alzheimer’s disease. These case-control studies lacked consideration for potential confounding. The animal evidence indicates that epidemiologic studies assessing concurrent brain Pb levels or occupational Pb exposure may not have examined the etiologically relevant exposure period. However, the observations that were made in experimental animals with high Pb exposure and blood Pb levels (40-100 µg/dL) may have uncertain relevance to this ISA. Further, animals were not behaviorally assessed for dementia.

4.3.9.2 Amyotrophic Lateral Sclerosis

The 2006 Pb AQCD (U.S. EPA, 2006b) reported mixed epidemiologic findings for an association between Pb and ALS based on case-control studies, several of which relied on

indirect methods of assessing Pb exposure. Case-control studies that measured blood Pb levels produced contrasting results. A study of 16 ALS cases (mean blood Pb level: 12.7 µg/dL) and 39 controls (mean blood Pb level: 10.8 µg/dL) found a small difference in the mean concurrent blood Pb level (Vinceti et al., 1997). A larger study of 109 cases and 256 controls that examined concurrent blood and bone Pb levels in a New England-area population found higher odds of ALS among subjects with concurrent blood Pb levels 3-14 µg/dL (e.g., OR: 14.3 [95% CI: 3.0, 69.3] for n = 55 with blood Pb levels 3-4 µg/dL compared with blood Pb levels <1-2 µg/dL) (Kamel et al., 2002). Odds of ALS were elevated in subjects in the highest two tertiles of patella and tibia Pb levels (≥ 10 µg/g patella Pb and ≥ 8 µg/g tibia Pb), but lacked precision. For example, compared with subjects with tibia Pb level <7 µg/g, the OR for tibia Pb levels 8-14 µg/g was 1.6 (95% CI: 0.5, 5.6). Results were adjusted for age, education, and hours/day inactive. Potential confounding by smoking was not considered. Also in this population, an estimate of cumulative Pb exposure based on occupational history was found to be associated with ALS (Kamel et al., 2002). The stronger findings for blood Pb level were surprising given that bone Pb level is a better biomarker of cumulative Pb exposure. One explanation for these findings is reverse causation. Blood was collected from people who already had ALS, and reduced physical activity among those with ALS could lead to more bone turnover and greater release of Pb from bones into circulation in ALS cases than controls.

Since the 2006 Pb AQCD, a few additional studies of ALS have been conducted with the same New England-area case-control group. Kamel et al. (2005) reported that the association between blood Pb level and ALS was not modified by the ALAD genotype, and Kamel et al. (2008) found that higher tibia and patella Pb levels were associated with longer survival time among 100 of the original 110 ALS cases with adjustment for age, sex, and ever smoking. Results were not altered by the additional adjustment for education, BMI, family history of ALS, or concurrent physical activity. Higher blood Pb levels were not associated with survival time. These paradoxical findings that point to a protective effect of Pb are not easily explainable but find coherence with results for Pb-induced increased survival time in an ALS mouse model (see below). On one hand, the cases with longer survival time may have higher bone Pb levels because they reflect a longer period of cumulative exposure. On the other hand, with longer survival time, there could be greater progression of disease and less mobility. Decreased mobility would tend to increase bone resorption, lower bone Pb levels, and increase blood Pb levels over time. The latter hypothesis is a less likely explanation for findings in this New England cohort because higher bone Pb levels were more strongly associated with longer survival time than was blood Pb level.

Another case-control study examined concurrent blood Pb levels and ALS among 184 cases (33 were either progressive muscular atrophy or primary lateral sclerosis, mean blood Pb level: 2.41 µg/dL) and 194 controls (mean blood Pb level: 1.76 µg/dL) (Fang et al., 2010). The cases were recruited from the National Registry of U.S. Veterans with ALS, and controls were recruited from among U.S. Veterans without ALS and frequency matched by age, gender, race, and past use of the Veterans Administration system for health care. A doubling of concurrent blood Pb level was associated with ALS with an OR of 1.9 (95% CI: 1.3, 2.7) with adjustment for age and a collagen protein as an indicator of bone formation. The association did not differ substantially by of the degree of bone turnover but was slightly higher among ALAD 1-1 carriers. The association with blood Pb level was similar in analyses that excluded the progressive muscular atrophy and primary lateral sclerosis cases. The similar results by degree of bone turnover suggest that reverse causation is not likely explaining the association between blood Pb level and ALS. However, as in other ALS case-control studies, the directionality of effects is difficult to establish. This study did not have measures of bone Pb to assess the association with biomarkers of longer-term Pb exposure.

Although epidemiologic studies have provided inconsistent evidence for associations of Pb biomarker levels with ALS in adults, toxicological studies have found that Pb exposure affects neurophysiologic changes associated with ALS. For example, chronic postnatal Pb exposure from weaning onward (200 ppm Pb acetate in drinking water, resultant blood Pb level: 27 µg/dL) reduced astrocyte reactivity and induced increased survival time in the superoxide dismutase transgenic (SOD1 Tg) mouse, which has SOD mutations found in humans with familial ALS (Barbeito et al., 2010). In this model, Pb exposure did not significantly increase the onset of the ALS disease. These findings provide coherence with the association observed between bone Pb level and longer survival time in patients diagnosed with ALS (Kamel et al., 2008).

Research outside of the Pb field has suggested different mechanisms for ALS initiation versus ALS progression, i.e., motor neuron function versus astrocyte and microglia function (Yamanaka et al., 2008; Boillee et al., 2006). Astrocyte vascular endothelial growth factor (VEGF) was examined for its involvement in the effects of Pb on increasing survival time in the ALS mouse model. Lower VEGF expression has been linked with risk of ALS in humans and ALS-like symptoms in animals. Baseline VEGF levels were elevated in astrocytes from the ventral spinal cord of untreated SOD1 Tg mice versus untreated nontransgenic mice. VEGF was not induced in the astrocytes of Pb-treated nontransgenic mice. In comparison, Pb-exposed SOD1 Tg mice, which had longer survival time, also had significant elevations in astrocyte VEGF (Barbeito et al., 2010). These findings for Pb-induced effects on astrocytes in a mouse model for ALS may provide a mechanistic explanation for Pb effects on increasing survival time in ALS.

Others reported that VEGF administration to the SOD1 Tg mice significantly reduced glial reactivity, a marker or neuroinflammation (Zheng et al., 2007). Using a cell-based co-culture system of neurons and astrocytes isolated from Pb-exposed SOD1 Tg mice, Barbeito et al. (2010) found that an up-regulation of VEGF production by astrocytes was protective against motor neuron death in the SOD1 Tg mouse cells. Thus, in vivo and in vitro results indicate that chronic Pb exposure resulted in increased survival time in an ALS mouse model and was correlated with higher spinal cord VEGF levels, which made astrocytes less cytotoxic to surrounding motor neurons (Barbeito et al., 2010).

In summary, there is inconsistent evidence of association between indicators of Pb exposure (history of occupational exposure, Pb biomarker levels) and ALS prevalence and survival time in humans. Other limitations include the potential for reverse causation and bias due to survival time in the case-control studies and the lack of objective assessment of occupational exposure. While several studies have considered potential confounding by age, education, and physical activity, few have considered smoking. Toxicological evidence also points to Pb exposure increasing survival in a mouse model of ALS and has suggested explanations including Pb-induced increases in VEGF expression and subsequent reduction in glial activity and protection of motor neurons against inflammation.

4.3.9.3 Parkinson's Disease

Previous Pb AQCDs reviewed a few studies, some ecological (Rybicki et al., 1993; Aquilonius and Hartvig, 1986) and some case-control relying on questionnaire data or occupational history (Gulson et al., 1999; Gorell et al., 1997; Tanner et al., 1989) that indicated associations between exposure to heavy metals, particularly Pb, and risk of Parkinson's disease. The limited number of previous studies, weak study designs, and weak Pb exposure assessment did not permit firm conclusions. Recent studies maintain several of these limitations but have indicated associations with bone Pb levels.

A recent large case-control study (330 cases, 308 controls) examined a population in the Boston, MA area with virtually no occupational exposures to Pb (Weisskopf et al., 2010). Subjects in the highest quartile of tibia Pb level (>16.0 μg/g) had higher odds of Parkinson's disease compared to those in the lowest quartile (≤ 3.1 μg/g) (OR: 1.91 [95% CI: 1.01, 3.60]) with adjustment for age, race, pack-years smoking, education, and recruitment site. Cases and controls were recruited from several different sources including movement disorder clinics and the NAS, which could have introduced biased participation by Pb exposure or reduced representativeness to the target population. In the NAS, cases were ascertained from self-report, which may introduce measurement error.

However, when analyses were restricted to cases recruited from movement disorder clinics and their spouse, in-law, or friend as controls, the results were even stronger (OR: 2.57 [95% CI: 1.11, 5.93] for tibia Pb >13.9 µg/g). Although the use of spouse, in-law, and friend controls can introduce bias, this is expected to be toward the null as these groups are likely to share many exposures. Weisskopf et al. (2010) did not adjust results for Mn exposure, which has been associated with Parkinsonian symptoms and could potentially confound associations between Pb and Parkinson's disease. However, unlike occupational exposure to Pb, environmental Pb exposure is less likely to be correlated with environmental Mn exposure. Thus, it is less likely that the associations observed with Pb were confounded by co-occurring Mn exposure.

Coon et al. (2006) conducted a smaller case-control study of 121 Parkinson's disease patients and 414 controls frequency-matched by age, sex, and race, all receiving health care services from the Henry Ford Health System in Michigan. Subjects in the highest quartile of both tibia (OR: 1.62 [95% CI: 0.83, 3.17] for levels ≥ 15 µg/g) and calcaneus (OR: 1.50 [95% CI: 0.75, 3.00] for levels ≥ 25 µg/g) bone Pb levels had higher but imprecise odds of Parkinson's disease compared to those in the lowest quartiles (0-6 µg/g for tibia and 0-12 µg/g for calcaneus). Subjects in the highest quartile of whole-body lifetime Pb level (≥ 81 µg/g, estimated using PBPK modeling) had the highest OR: 2.27 (95% CI: 1.13, 4.55) versus the lowest quartile, 0-40 µg/g. These results were adjusted for age, race, sex, pack-years smoking, regular coffee consumption, and regular alcohol use, but Mn exposure was not considered. It was not clear what the extent of occupational exposure to Pb was among the participants; however, a previous Henry Ford Health System study had linked occupational Pb exposure to Parkinson's disease (Gorell et al., 1997). Thus, it is uncertain whether the observed associations were confounded by co-occurring Mn exposure.

In summary, a few recent case-control studies expand on previous evidence by finding associations of tibia and calcaneus bone Pb levels, biomarkers of cumulative Pb exposure, with Parkinson's disease in adults. The associations observed with biomarkers of cumulative Pb exposure increase confidence that associations are not explained by reverse causation. However, firm conclusions are not warranted. While associations were adjusted for potential confounding by age, sex, race, and education, Mn co-exposure was not considered.

4.3.9.4 Essential Tremor

The few available case-control studies of essential tremor have found associations with concurrent blood Pb levels. The 2006 Pb AQCD (U.S. EPA, 2006b) described case-control studies that found associations between concurrent blood Pb levels and essential tremor in New York City metropolitan area populations (Louis et al., 2005; Louis et al., 2003). In the larger study, mean (SD) blood Pb levels were 3.3 (2.4) µg/dL in the 100 essential tremor cases and 2.6 (1.6) µg/dL in the 143 controls (Louis et al., 2003). In the other study, mean (SD) blood Pb levels were 3.5 (2.2) µg/dL in the 63 essential tremor cases and 2.6 (1.5) µg/dL in the 101 controls (Louis et al., 2005). In Louis et al. (2005), the magnitude of association was larger among the 35 ALAD-2 carriers than among 129 adults homozygous for ALAD-1. History of occupational Pb exposure was similarly rare in cases and controls (2%).

Recently, Dogu et al. (2007) reported on 105 essential tremor cases selected from a movement disorder clinic in Turkey and 105 controls (69 spouses and 36 other relatives) living in the same district. With adjustment for age, sex, education, smoking status, cigarette pack-years, and alcohol use, a 1 µg/dL higher blood Pb level (measured at the time of study recruitment) was associated with essential tremor with an OR of 4.19 (95% CI: 2.59, 6.78). This OR was much larger than that obtained in the New York area study (OR: 1.19 [95% CI: 1.03, 1.37]) (Louis et al., 2003). The magnitude of association in Dogu et al. (2007) is even more striking because so many of the controls were spouses who are expected to share many environmental exposures as cases. Most of the essential tremor cases were retired at the time of the study; however past occupational history was not examined.

In summary, a small body of studies indicates associations between blood Pb level measured at the time of the study and prevalence of essential tremor in adults. However, because of the case-control design, reverse causation cannot be excluded as a potential explanation for the observed associations since loss of physical activity and subsequent bone resorption may lead to an increase in blood Pb level. Further, the level, timing, frequency, and duration of Pb exposure associated with essential tremor are uncertain. History of occupational Pb exposure was not consistently examined, and potential confounding by Mn exposure was not examined.

4.3.9.5 Toxicological Studies of Cell Death Pathways

A common element of the neurodegenerative diseases described above is neuronal cell death. Studies reviewed in the 2006 Pb AQCD (U.S. EPA, 2006b) documented that Pb exposure induced cell death or apoptosis in various models including rat brain (Tavakoli-Nezhad et al., 2001), retinal rod cells (He et al., 2003; He et al., 2000), and cerebellar neurons (Oberto et al., 1996). Recent studies produced similar findings, in most cases, in animals with higher blood Pb levels than those relevant to this ISA. Long-term (40 days) exposure to 500 ppm Pb in drinking water was found to increase pro-apoptotic Bax protein levels and the number of apoptotic cells in the hippocampus in young (exposure starting at 2-4 weeks of age) and adult (exposure starting at 12-14 weeks of age) male rats with blood Pb levels 91 and 98 µg/dL (Sharifi et al., 2010). Apoptosis was verified by light and electron microscopy. These investigators found Pb-induced apoptosis of PC12 neuronal cells in vitro (Sharifi and Mousavi, 2008). Another study followed the developmental profile of changes in various apoptotic factors in specific brain regions of rats exposed to 2,000 ppm Pb acetate during lactation (to PND20) via drinking water of dams (Chao et al., 2007). At PND20, male offspring blood Pb level was 80 µg/dL. Pb exposure significantly upregulated hippocampal mRNA for various apoptotic factors including caspase-3, Bcl-x, and Brain-derived neurotrophic factor (BDNF) on PND12, PND15 and PND20. The cortex of these male pups showed upregulation of Bcl-x and BDNF on PND20 and PND25. The cerebellum did not have elevated apoptotic mRNA levels in this model. Thus, in this study, Pb-induced apoptosis varied by age and brain region in male offspring.

Pb exposure also has been shown to induce apoptosis of spinal cord cells during spinal cord development in chicks treated with 150 or 450 µg Pb acetate in ovo at embryonic day 3 or 5 and visualized six days later (Müller et al., 2012). TUNEL positive cells, indicating DNA fragmentation induced by apoptosis, were at significantly higher levels in Pb-exposed animals and were visualized in all layers of the developing spinal cords. Also, levels of glial fibrillary acidic protein (GFAP), a factor important in neuronal migration and cellular differentiation during nervous system development, was significantly attenuated in spinal cords of Pb-exposed chicks. Liu et al. (2010b) examined apoptotic effects in 30 day-old male rats that were treated with Pb acetate once daily for 6 weeks via intragastric infusion. Four groups: control, low (2 mg/kg BW), medium (20 mg/kg BW), and high (200 mg/kg BW) had blood Pb levels of 1-7.5 µg/dL; 4.5-11.4 µg/dL; 8.9-42 µg/dL; and 48-73 µg/dL, respectively. Pb induced hippocampal neuronal apoptosis (TUNEL positive staining, statistically significant at all Pb doses) and downregulation of hippocampal XIAP (significant at high dose only) and Smac (statistically nonsignificant trend) at the termination of the 6 week treatment. In another study, Pb exposure (500 ppm Pb acetate in drinking water for 8 weeks) of adult male rats

induced regional-specific changes in brain apoptotic proteins poly(ADP-ribose) polymerase, Bcl-2, and caspase-3 with a greater effect observed in the hippocampus and cerebellum than the brainstem or frontal cortex (Kiran Kumar et al., 2009).

In summary, a small body of epidemiologic studies found Pb-associated increases in essential tremor and Parkinson's Disease in adults. However, the implications of these findings are limited because of the potential for reverse causation to explain cross-sectional associations observed with blood Pb level, and the potential for confounding by Mn exposure. However, toxicological evidence supports an effect of Pb on neurodegeneration by demonstrating that Pb exposure during various lifestages, early postnatal or adulthood, induces neuronal apoptosis in animals. Several of these observations were made with routes (i.e., i.p.) or concentrations of Pb exposure that may not be relevant to this ISA.

4.3.10 Modes of Action for Pb Nervous System Effects

4.3.10.1 Effects on Brain Physiology and Activity

The 2006 Pb AQCD (U.S. EPA, 2006b) reviewed a small body of available epidemiologic studies demonstrating associations of Pb biomarkers with electrophysiologic and physical changes in the brains of young adults (Yuan et al., 2006; Cecil et al., 2005) and children (Meng et al., 2005; Trope et al., 2001) as assessed by magnetic resonance imaging (MRI) or spectroscopy (MRS). The implications of previous findings were limited by the small sample sizes (n = 12-45) and limited consideration for potential confounding. Recent studies examining MRI data were limited largely to the Cincinnati cohort as adults (ages 19-24 years). In addition to supporting associations of childhood blood Pb levels with physiological changes in the brain of adults, these recent analyses expanded on previous studies by including larger sample sizes, aiming to characterize potentially important lifestages of Pb exposures, and evaluating potential links between changes in brain activity and functional neurodevelopmental effects. While overall there are few studies in few populations, by showing physical and physiologic changes in areas of the brain associated with neurodevelopmental function, the evidence provides biological plausibility for the associations observed between Pb biomarker levels and cognitive function and behavior.

In prospective analyses of the Cincinnati cohort as adults (ages 20-23 years, n = 35, 42), Cecil et al. (2005) and Yuan et al. (2006) conducted functional MRI during a verb generation language task and found that higher age 3-78 month average blood Pb level (mean 14.2 µg/dL) was associated with decreased activation in the left frontal gyrus and

left middle temporal gyrus, regions implicated in semantic language function. Yuan et al. (2006) considered birth weight, marijuana consumption, sex, SES, gestational age, and IQ as potential confounding factors. Whereas previous analyses of the Cincinnati cohort focused on activity in specific regions of the brain, Cecil et al. (2011) examined brain metabolites. Higher age 3-78 month average blood Pb levels (mean: 13.3 µg/dL) were associated with lower levels of N-acetylaspartate (NAA) and creatine (Cr) in the basal ganglia and lower levels of choline in white matter in 159 adults, ages 19-23 years. These results were adjusted for age and FSIQ; however, several other unspecified factors were considered. Lower levels of NAA, Cr, and choline are linked to decreased neuronal density and alteration in myelin. A recent prospective analysis of 31 men in the NAS cohort similarly reported an association between biomarkers of cumulative, long-term Pb exposure and changes in brain metabolites in older adults. Weisskopf et al. (2007a) found higher tibia and patella Pb levels to be associated with a higher myoinositol/Cr ratio in the hippocampus measured more than 10 years after bone Pb was measured and adjusted for age. Myoinositol/Cr ratio may be indicative of glial activation and is a signal reportedly found in the early stages of HIV-related dementia and Alzheimer's disease.

Other studies in the Cincinnati cohort as young adults found that childhood average blood Pb levels were associated with altered brain architecture. Among 91 adults ages 20-26 years, Brubaker et al. (2009) found associations of age 3-78 month average blood Pb levels (mean: 12.9 µg/dL) with diffusion parameters that were indicative of less organization of fibers throughout white matter. Results were adjusted for maternal IQ, prenatal alcohol and tobacco exposure, and adult marijuana use. In regions of the corona radiata, higher blood Pb levels were associated with less myelination axonal integrity. In regions of the corpus callosum, higher blood Pb levels were associated with greater myelination and axonal integrity. The differential impact among neural elements may be related to the stage of myelination development present at various time periods.

Another study of 157 Cincinnati cohort adults ages 19-24 years provided evidence of region-specific reductions in gray matter volume in association with age 3-78 month average blood Pb levels (mean: 13.3 µg/dL) with adjustment for sex (Cecil et al., 2008). The most affected regions included frontal gray matter, specifically the anterior cingulate cortex, and the ventrolateral prefrontal cortex (i.e., areas related to executive functions, mood regulation, and decision-making). Further, fine motor factor scores were positively correlated with gray matter volume in the cerebellar hemispheres; adding blood Pb level as a variable to the model attenuated this correlation. These findings suggested that changes observed with MRI may mediate the association between blood Pb levels and decrements in motor function. The functional relevance of these structural changes in the brain also is supported by observations from other studies that link changes in brain architecture and activity to changes in cognitive function (e.g., visuoconstruction, visual

memory, eye-hand coordination) (Schwartz et al., 2007) and behavioral problems (impulsivity, aggression, violence) (Yang et al., 2005; Raine et al., 2000). In a subsequent comparison of blood Pb levels measured at various lifestages in 157 Cincinnati cohort adults ages 19-24 years, Brubaker et al. (2010) found that higher blood Pb levels at older ages (annual means from ages 3-6 years, means: 9.6-16.3 µg/dL) were associated with greater losses in gray matter volume than were age 1 or 2 year (means: 10.6, 17.2 µg/dL), 3-78 month average or maximum blood Pb levels (mean: 23.1 µg/dL). Both Cecil et al. (2008) and Brubaker et al. (2010) found that Pb-associated reductions in gray matter were more pronounced in males than females in the Cincinnati cohort.

Studies of Pb-exposed workers (n = 15-532) also found associations of concurrent blood (means: 17-63.5 µg/dL) and tibia (mean 14.5 µg/dL) Pb levels with changes in brain structure and physiology, supporting the effects of chronic Pb exposure. Pb-associated changes included white matter lesions, smaller brain volumes, less total gray matter, and lower levels of brain metabolites such as NAA and Cr (Hsieh et al., 2009b; Jiang et al., 2008; Bleecker et al., 2007b; Stewart et al., 2006) with adjustment for similar factors as associations for cognitive function. Other occupational exposures were not examined. In a few of these occupational groups, Pb-associated brain changes were linked to poorer performance in cognitive function tests (Caffo et al., 2008; Bleecker et al., 2007b).

Higher concurrent blood Pb level also was associated with lower NAA/Cr ratio in small cross-sectional studies that included children (n = 6, 16, ages 4-21 years), although neither study considered potential confounding (Meng et al., 2005; Trope et al., 2001). All subjects had normal MRIs with no evidence of structural abnormalities. Thus, the biological relevance of the observed physiological changes is unclear. Additionally, the representativeness of findings is uncertain because results were based on comparisons of subjects with relatively high blood levels (means: 38, 40 µg/dL) to those with blood Pb levels <10 µg/dL.

In summary, results in a few populations indicate associations of childhood blood or adult tibia Pb levels with changes in brain structure and physiology in adults assessed by MRI or MRS. Associations were found in children, but implications are more limited because of small samples sizes, lack of consideration of potential confounding, and high blood Pb levels of the children examined. Evidence from the prospective Cincinnati cohort studies improves characterization of the temporal sequence between Pb exposure and changes in brain structure and physiology. Several studies linked these changes to functional changes in cognitive performance or motor skills. Because of the small samples sizes of several studies and limited consideration for potential confounding, implications of the effects of Pb exposure specifically on changes in brain structure and physiology are not

certain. However, the evidence provides biological plausibility for the associations observed between Pb biomarker levels and cognitive function and behavioral problems.

4.3.10.2 Oxidative Stress

Because the brain has the highest energy demand and metabolism of any organ, energy homeostasis is of utmost importance. Energy imbalance can increase the susceptibility of the highly energetic brain tissue to stressors and cell death. Pb has been shown to induce energy imbalance by inhibiting various enzymes involved in energy production or glucose metabolism including glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate kinase, and succinate dehydrogenase (Verma et al., 2005; Yun and Hoyer, 2000; Regunathan and Sundaresan, 1984; Sterling et al., 1982). Mitochondria produce ATP or energy through oxidative phosphorylation. Aberrant mitochondrial function can decrease the energy pool and contribute to ROS formation via electron transport chain disruption. ATP depletion can also affect synaptic and extracellular neurotransmission. The mitochondrial Na^+/K^+ATPase is important in maintaining the inner mitochondrial membrane potential $\Delta\Psi_m$ (delta psi[sub m]) and the functioning of the mitochondria.

Gestational Pb exposure of mice was found to impair mitochondrial function and energy production in neuronal cells and produce concomitant increases in mitochondrial and cellular ROS production. In these mice, 1,000 ppm Pb acetate in dam drinking water resulted in offspring blood Pb levels of 4 µg/dL and cerebella Pb levels of 7.2 µg/g dry weight) (Baranowska-Bosiacka et al., 2011b). Cerebellar granular cells were harvested from control and Pb-exposed animals at PND8. These neuronal cells were cultured for 5 days in vitro, at which point various mitochondrial parameters were measured. With Pb exposure, ROS were significantly increased in both the cortical granule cells and in the mitochondria. Intracellular ATP concentration and adenylate energy charge values were significantly decreased in cells of Pb-exposed mice versus controls. Neuronal Na^+/K^+ATPase activity was significantly lower in cortical granule cells from Pb-exposed mice versus cells from controls. Mitochondrial mass was unaffected with Pb treatment, but mitochondrial membrane potential was significantly decreased with Pb exposure. Energy imbalances also were found in Wistar rats (PND15) of each sex injected daily for 2 weeks with Pb acetate (15mg/kg BW, i.p., resulting in a mean blood Pb level of 31 µg/dL; control blood Pb level 3 µg/dL) (Baranowska-Bosiacka et al., 2011a). ATP and ADP were significantly decreased in various brain regions with Pb exposure, with the cerebellum and hippocampus more strongly affected than the forebrain cortex. Also, expression of the pro-inflammatory P2XR receptor was enhanced in the glial fraction, indicating the astrocyte pool may be involved in the pathological changes found in Pb-exposed immature rat brains. Mitochondrial energy imbalances also were found in

Pb-exposed crayfish that were placed under hypoxic conditions which induced a decrease in metabolism (Morris et al., 2005).

In rats, Pb exposure has been shown to induce oxidative stress, in some cases, with concomitant functional CNS changes. Exposure of adult male rats to 4,000 ppm Pb acetate in drinking water for 6 weeks increased brain levels of lipid peroxides (LPO) and lowered levels of antioxidants including nitric oxide (NO), total antioxidant capacity (TAC), glutathione (GSH), glutathione-S-transferase (GST), and superoxide dismutase (SOD). In red blood cells, Pb levels were positively correlated with LPO levels and negatively correlated with NO levels (Hamed et al., 2010). These effects of Pb on oxidative stress parameters were attenuated with co-exposure to green tea extract (1.5%), which reduced brain (1.9 to 1.2 ppm) and blood Pb levels of rats (0.773 to 0.654 ppm). In a study of adult male Wistar albino rats, Pb acetate treatment by i.p. (20 mg/kg daily, 5 days) elevated lipid peroxidation, neuronal damage, and brain tissue DNA fragmentation and decreased antioxidant GSH levels and antioxidant enzyme activity, (Abdel Moneim et al., 2011a). These effects were attenuated with co-administration of the polyunsaturated fatty acid flaxseed oil (oral gavage 1,000mg/kg body weight for 5 days, 1 hour prior to Pb dosing). Flaxseed oil co-treatment also was associated with significantly lower blood Pb level in Pb-exposed animals and control animals, indicating that flaxseed oil may alter Pb toxicokinetics in animals. Another study provided indirect evidence of Pb-induced oxidative stress with observations that Pb-induced (2,000 ppm Pb acetate in drinking water PND1-PND67) impairments in long-term potentiation (LTP), paired-pulse reactions, and input/output functions in the DG of male and female Wistar rats were significantly attenuated with co-treatment with the antioxidant quercetin (i.p. 30 mg/kg BW, PND60-PND67) at PND67 (Hu et al., 2008a). Quercetin-treated animals had significantly less hippocampal Pb than did the animals exposed only to Pb.

Oxidative stress may be involved in neurodegenerative pathologies including Alzheimer's disease. Hydrogen peroxide-induced oxidative stress has been shown to induce intracellular accumulation of Ab in human neuroblastoma cells (Misonou et al., 2000). Oxidative stress-induced DNA damage can be measured as the ratio of the adduct 8-hydroxy-2’-deoxyguanosine to 2-deoxyguanosine (8-oxo-dG/2-dG). 2-dG is a product of oxidative cleavage and is oxidized to form 8-oxo-dG. Pb-induced changes in the 8-oxo-dG to 2-dG ratio were examined recently as a mechanism underlying neurodegeneration. Similar to Ab levels, changes in the 8-oxo-dG to 2-dG ratio showed a biphasic relationship in the brains of rats exposed to 2,000 ppm Pb acetate via drinking water of dams from PND1-PND20 (blood Pb level 46 µg/dL) (Bolin et al., 2006). The 8-oxo-dG to 2-dG ratio decreased early in exposure (PND5) but increased at age 20 months. No increase was found in animals exposed to Pb from age 18 to 20 months (blood Pb level: 60 µg/dL). Activity of the base-excision DNA repair enzyme 8-oxo-

guanine DNA glycosylase was unaffected by Pb exposure. Similar findings were reported in a monkey study (Wu et al., 2008a). The ratio of 8-oxo-dG to 2-dG in the brains of aged monkeys (23 years) was significantly elevated above that in controls with Pb exposure only in infancy (PND1-PND400, infant formula, blood Pb levels: 19-26 µg/dL at PND400) (Wu et al., 2008a). Thus, evidence in rats and monkeys suggests a possible role for oxidative stress in Pb-induced neurodegenerative effects and indicates that early life Pb exposure induces oxidative DNA damage and amyloidogenesis in aged animals.

4.3.10.3 Nitrosative Signaling and Nitrosative Stress

The NO system is increasingly being recognized as a signaling system in addition to its more classical role as a marker of cellular stress. Pb exposures during the gestational-early postnatal period (GD6-PND21) (Chetty et al., 2001) and after lactation only (Fan et al., 2009a) were found to reduce hippocampal levels of NO or neuronal NO synthase. In the hippocampus, NO mediates LTP, which is considered to be a major cellular mechanism underlying learning and memory. Thus, observations of Pb-induced changes in hippocampal NO may provide a mechanistic explanation for the effects of Pb on cognitive function decrements. Fan et al. (2009a) found reduced hippocampal NOS and NO in weanling male rats after either 4 or 8 weeks of Pb exposure resulting in blood Pb levels of 48 and 70 µg/dL, respectively. In the same study, dietary supplementation with taurine or glycine concomitant with 8 weeks of Pb exposure induced significant increases in hippocampal NOS, whereas Pb plus dietary supplementation with vitamin C, methionine, tyrosine, or vitamin B1 decreased hippocampal NOS. In this study, co-exposure of specific nutrients also prevented Pb-induced impairments in learning as evidenced by lack of increased escape latency in the Morris water maze. Dietary supplementation with tyrosine, methionine, or ascorbic acid after 4 weeks of Pb exposure (blood Pb levels upon cessation of exposure and after 4-week lag: 48 and 8.1 µg/dL, respectively) reversed Pb-induced decrements in NO/NOS. Zn supplementation given after Pb exposure had no effect on the NO system.

4.3.10.4 Synaptic Changes

Previous toxicological studies pointed to an effect of developmental Pb exposure on synapse development, which mechanistically may contribute to multiple Pb-related aberrant effects, including changes in LTP and facilitation. Facilitation of a neuronal terminal is defined as the increased capability to transmit an impulse down a nerve due to prior excitation of the nerve. Earlier work showed that Pb exposure resulted in altered density of dendritic hippocampal spines (Király and Jones, 1982; Petit and Leboutillier,

1979), reduced synapse formation (Chen et al., 2005b), and abnormal long-term plasticity (Nihei et al., 2000). In a recent study, Li et al. (2009c) focused on inflammatory endpoints and synaptic changes after gestational plus lactational Pb exposure (1,000-10,000 ppm Pb acetate via drinking water of dams, producing offspring blood Pb levels 40-100 µg/dL, respectively at PND21). Hippocampal TNF-α was significantly elevated with Pb exposure, and proteins that comprise the SNARE complex were all changed with Pb exposure. The SNARE complex of synaptic proteins includes SNAP-25, VAMP-2 and Syntaxin 1a and is essential in exocytotic neurotransmitter release at the synapse. Thus, Li et al. (2009c) found significant differences in hippocampal synaptic protein composition and increased pro-inflammatory cytokine levels in the brains of Pb-exposed offspring.

Recent research using the Drosophila larval neuromuscular junction model showed that compared with unexposed controls, Pb-exposed larvae had significant increases in intracellular calcium and significant delays in calcium decays back to baseline levels at the pre-synaptic neuronal bouton (as stimulated with multiple action potentials, also called AP trains). Pb-exposed larvae had reduced activity of the plasma membrane Ca^{2+}ATPase, which is responsible for extravasations of calcium from the synaptic terminal (He et al., 2009). Intracellular calcium in Pb-exposed larvae was no different from that in controls under resting conditions or in neurons with stimulation by a single action potential. Pb media concentrations in these experiments were 100 or 250 µM with the body burden of Pb from the lower dose estimated to be 13-48 µM per larvae. After stimulation of the axon, facilitation of the excitatory post-synaptic potential, which is dependent on residual terminal calcium, was significantly elevated in Pb-exposed larvae versus control (He et al., 2009). The data from this synapse study demonstrate that developmental Pb exposure affected the plasma membrane Ca^{2+}ATPase, induced changes in the intracellular calcium levels during impulse activation, and produced changes in facilitation of the neuronal networks of Drosophila. Thus, the neuromuscular junction is a potential site of Pb interaction.

Neurotransmission is an energy-dependent process as indicated by the presence of calcium-dependent ATP releases at the synaptic cleft. At the synapse, ATP is metabolized by ectonucleotidases. Acute exposure (96 hours) of male and female zebrafish to 2 µg/dL Pb acetate in their water induced significant decreases in ATP hydrolysis in brain tissue (Senger et al., 2006). This dose is deemed to be environmentally relevant. With chronic exposure (30 days), Pb acetate promoted the inhibition of ATP, ADP and AMP hydrolysis. These findings were consistent with findings in rodents (Baranowska-Bosiacka et al., 2011a). The authors hypothesized that at 30 days, this Pb-induced change in nucleotide hydrolysis was likely due to post-translational modification because expression of enzymes responsible for the hydrolysis,

NTPDase1 and 5′-nucleotidase, were unchanged (Senger et al., 2006). Thus, Pb was shown to affect nucleotidase activity in the CNS of zebrafish, possibly contributing to aberrant neurotransmission.

Another enzyme important in synaptic transmission at cholinergic junctions in the CNS and at neuromuscular junctions is acetylcholinesterase (AChE). After 24 hours of exposure to 2 µg/dL Pb acetate in water, AChE activity was significantly inhibited in zebrafish brain tissue (Richetti et al., 2010). AChE activity returned to baseline by 96 hours and maintained baseline activity after 30 days of exposure. Thus, Pb was shown to affect synaptic homeostasis of AChE in the brains of zebrafish only transiently.

Pb has been shown to act as an antagonist of the NMDA receptor (NMDAR). The NMDAR is essential for proper presynaptic neuronal activity and function. Primary cultures of mouse hippocampal cells exposed to 10 or 100 µM Pb during the period of synaptogenesis had loss of two proteins necessary for presynaptic vesicular release, synaptophysin (Syn) and synaptobrevin (Syb) but no change in a similar protein synaptotagmin (Syt) (Neal et al., 2010a). This deficit was found in both GABAergic and glutamatergic neurons. Pb also induced an increase in the number of presynaptic contact sites. But, these sites may have been nonfunctional as they lacked the protein receptor complexes necessary for proper vesicular exocytosis. Another factor involved in maturation and signaling of presynaptic neurons is BDNF, which is synthesized and released by postsynaptic neurons and regulated by the NMDAR. In mouse hippocampal cells, both pro-BDNF and BDNF release were significantly attenuated with Pb exposure (Neal et al., 2010a). Further, exogenous BDNF administration rescued the aforementioned Pb-related effects on presynaptic proteins. Thus, results from this cell culture model showed that Pb-related presynaptic aberrations are controlled by NMDAR-dependent BDNF effects on synaptic transmission.

Glutamate is another neurotransmitter that is released from presynaptic neurons and via interactions with the NMDAR causes postsynaptic neuron depolarization. A recent study of Wistar albino rats exposed to Pb postnatally from birth to age 12 weeks (drinking water 3×10^4 µg/dL Pb acetate, resulting in blood Pb levels of 17 µg/dL at age 6 weeks) showed decreased learning ability, decreased hippocampal glutamate at 6, 8, 10 and 12 weeks of age, as well as significant decrements in the hippocampal glutamate synthesis-related enzymes aspartate aminotransferase and alanine aminotransferase (Niu et al., 2009).

4.3.10.5 Blood Brain Barrier

Two barrier systems exist in the body to separate the brain or the CNS from the blood: the blood brain barrier (BBB) and the blood cerebrospinal fluid barrier (BCB). The BBB, formed by tight junctions at endothelial capillaries forming the zonulae occludens (occludins, claudins, and cytoplasmic proteins), separates the brain from the blood and its oncotic and osmotic forces, allowing for selective transport of materials across the BBB.

Pb exposure during various developmental windows has been shown to increase the permeability of the BBB of animals (Dyatlov et al., 1998; Struzynska et al., 1997b; Moorhouse et al., 1988; Sundstrom et al., 1985). Possibly due the underdevelopment of the BBB early in life, prenatal and perinatal Pb exposure has been found to result in higher brain Pb accumulation than have similar exposures later in life (Moorhouse et al., 1988). The choroid plexus and cerebral endothelial cells that form the BBB and BCB tight junctions have been shown to accumulate Pb more than other cell types and regions of the CNS. Studies reviewed in the 2006 Pb AQCD showed that the chemical form of Pb and its capability to interact with proteins and other blood components affect its capability to penetrate the BBB (U.S. EPA, 2006b). Pb also has been shown to compromise the BCB and decrease the cerebrospinal fluid level of transthyretin, which binds thyroid hormone in the cerebrospinal fluid. Low thyroid hormone levels in pregnant women have been linked with IQ deficits in their children (Lazarus, 2005).

Recent research with male weanling rats exposed to Pb acetate via drinking water showed leaky cerebral vasculature, an indication of a compromised BBB, as detected histologically with lanthanum nitrate staining of the brain parenchyma (Wang et al., 2007b). Cerebral vasculature leakiness was ameliorated or resembled that of controls after Fe supplementation. The cerebral vasculature leakiness may by explained by observations of significant Pb-induced decreases in the BBB tight junction protein occludin in the hippocampus, brain cortex, and cerebellum of these weanling animals. Occludin levels were rescued to control levels with Fe supplementation. This loss of integrity at the junctional protein level was affirmed with additional experiments using the rat brain vascular endothelial cell line RBE4, in which 10 μM Pb acetate exposure for 2, 4, 8, 16 and 24 hours resulted in decreases in junctional proteins occludin and claudin 5 as well as scaffold proteins ZO1 and ZO2 (Balbuena et al., 2011). Because gene expression for these junctional and scaffold proteins did not show decrements, it was determined that these protein decrements were due to post-translational modifications.

Pb exposure also was found to contribute to leakiness of the BBB by decreasing the resistance across the junction (Balbuena et al., 2010). An in vitro co-culture system employing endothelial cells (RBE4 or bovine brain microvascular endothelial cells) and astrocytes (primary Sprague-Dawley neonatal pup astrocytes, GD21) served as the barrier

between Pb-containing media and neurons. Pb acetate exposure (1 and 10 µM) for 14 hours significantly impaired transendothelial electrical resistance, a marker of BBB integrity, in a concentration-dependent manner.

Long-term Pb exposure of adult mice was found to increase regional edema and BBB permeability (López-Larrubia and Cauli, 2011). Adult male rats exposed to Pb acetate in drinking water for 4 or 12 weeks (50 or 500 ppm, resulting in blood Pb levels of 12 and 55 µg/dL, respectively) were assessed by diffusion weighted imaging for changes in apparent diffusion coefficent (ADC), a measure of tissue water diffusivity that changes under pathological conditions like cerebral edema. With 12 weeks of exposure, 50 ppm Pb increased the ADC values in the cerebellum and mesencephalic reticular formation, and 500 ppm Pb exposure significantly increased ADC in the corpus callosum and caudate putamen. With 4 weeks of exposure, 500 ppm Pb significantly increased the water ADC in the hippocampus, mesencephalic reticular formation, and cerebellum but not in other brain areas. The brain areas with elevated ADC also showed increased BBB permeability as measured with evans blue dye.

4.3.10.6 Cell Adhesion Molecules

Classic cell adhesion molecules including neural cell adhesion molecule (NCAM) and the cadherins are junctional or cell surface proteins that are critical for cell recognition and adhesion. While direct effects of Pb on cell adhesion molecules have not been described, the calcium-dependency of these molecules suggests possible interactions with competing cations like Pb and a possible contribution to Pb effects on CNS barrier function disruption, neurite outgrowth, synaptic plasticity, learning, and memory (Prozialeck et al., 2002).

4.3.10.7 Effects on Glial Cells

Astroglia and oligodendroglia are supporting cells in the nervous system that maintain the extracellular space in the brain and provide structural support to neurons, deliver nutrients to neurons, and promote myelination. Glial cells provide immune surveillance in the brain and contribute to inflammation-mediated pathologies. In Wistar rats, Pb treatment (15 mg/kg of Pb acetate, i.p.) during early postnatal maturation was observed to produce chronic glial activation with inflammation and neurodegeneration (Strużyńska et al., 2007). Among the cytokines detected in the brains of these Pb-treated rats were IL-1β, TNF-α and IL-6. Glial cells have been shown to serve as Pb sinks in the developing and mature brain by sequestering Pb (Tiffany-Castiglioni et al., 1989). This

glial sequestration of Pb has been accompanied by a decrease in brain glutamine and a reduction in glutamine synthetase activity in the astroglia; astroglia take up released glutamate and convert it to glutamine. Pb has been shown to induce hypomyelination and demyelination (Coria et al., 1984) mediated through the oligodendrocytes with younger animals found to be more susceptible to the effects of Pb (Tiffany-Castiglioni et al., 1989). Pb accumulation in young glial cells may contribute to a lifelong exposure of neurons to Pb as Pb is released from the sink over time. Thus, Pb accumulation in glial cells can contribute to continual damage of surrounding neurons (Holtzman et al., 1987).

Glial transmitters

Evidence indicates that glial transmission is affected with Pb exposure and that the NMDAR may be involved in this aberrant glial transmission. To determine the contribution of the gliotransmitter serine to Pb-mediated changes in LTP, Sun et al. (2007) exposed rats to Pb acetate from gestation through lactation to PND28 via maternal drinking water and collected hippocampal sections. CA1 section LTPs were examined using in vitro patch clamp monitoring. Chronic Pb exposure impaired the magnitude of hippocampal NMDAR-dependent LTPs, but the magnitude of the LTPs was restored with supplementation with D-serine (Sun et al., 2007), which is an NMDAR agonist (Bear and Malenka, 1994). The use of 7-chlorokynurenic acid, an antagonist of the glycine binding site of the NMDAR, which also is the binding site of D-serine, effectively abolished the rescue of LTP by D-serine. NMDAR-independent LTP hippocampal neurotransmission was inhibited in slices of Pb-exposed mossy-CA3 synapses and was not rescued by exogenous D-serine supplementation.

4.3.10.8 Neurotransmitters

Pb has been shown to compete with calcium for common binding sites and second messenger activation. When Pb activates a calcium-dependent system in the nervous system, it can contribute to aberrant neurotransmitter regulation and release because this system intimately relies on calcium signaling for its homeostasis. Pb also has been shown to interfere with other physiological divalent cations. Pb-related alterations in neurotransmission are discussed in further detail below.

Monoamine Neurotransmitters and Stress

The monoamine neurotransmitters include dopamine (DA), serotonin (5HT), and norepinephrine (NE). Combined exposures of maternal stress and Pb exposure can

synergistically enhance neurobehavioral impairments in offspring and can sometimes potentiate an effect that would otherwise be sub-threshold. Virgolini et al. (2008a) found enhanced DA and NE release in male rats and enhanced NE release in female rats after developmental Pb exposure (50 or 150 ppm in drinking water, 2 months prior to mating through lactation, resulting in blood Pb levels of 11 µg/dL and 35 µg/dL, respectively) and combined maternal and offspring stress. In most cases, stress potentiated the effects of Pb exposure on offspring NE and DA concentrations. Regional 5HT levels were unaffected in offspring with Pb exposure alone. Pb (50 and 150 ppm) combined with stress (maternal and/or offspring) significantly potentiated 5HT levels in the frontal cortex in females and in the nucleus accumbens (NAC) and striatum in male offspring. The concentration of 5-Hydroxyindoleacetic acid (5HIAA), the main metabolite of 5HT, was significantly increased in the striatum of male offspring with 150 ppm Pb exposure alone. With 50 ppm Pb, stress potentiated striatal and frontal cortex 5HIAA in males. Potentiation of 5HIAA levels in females was significant in the NAC with 50 ppm Pb exposure; stress alone also significantly increased 5HIAA levels in the NAC of females with no Pb exposure. Pb-induced changes in brain neurochemistry with or without concomitant stress exposure are complex with differences varying by brain region, neurotransmitter type, and sex of the animal.

Monoamine Neurotransmitters and Auditory Function

Earlier work showed that perinatal Pb exposure of rats induced increased tyrosine hydroxylase, DA, and cerebral cortex catecholamine neurotransmission (Devi et al., 2005; Leret et al., 2002; Bielarczyk et al., 1996). Earlier publications examining various time windows, durations, and doses of Pb exposure indicated varying effects on monoamine neurotransmitters. In recent work, these neurotransmitters, among others, have been implicated in Pb effects on auditory function in various integration centers of the brainstem including the lateral superior olive (LSO) and the superior olivary complex (SOC). Among various functions, the SOC is vital for sound detection in noisy settings. A recent study in mice found significant decreases in immunostaining of LSO and SOC brainstem sections for monoamine vesicular transporter VMAT2, 5HT, and dopamine beta-hydroxylase (DbH, a marker for NE) after gestational-lactational Pb exposure (10 or 100 µM Pb acetate from the formation of breeding pairs to PND21) (Fortune and Lurie, 2009). This exposure period corresponds to the period of auditory development in the mouse. Statistically significant decreases in VMAT2 and DbH were found in mice with blood Pb levels of 8.0 and 42 µg/dL; however, decrements in 5HT were statistically significant only in mice with 8.0 µg/dL blood Pb level. Immunostaining for tyrosine hydroxylase and transporters including VGLUT1, VGAT, VAChAT indicated that they were unaffected by developmental Pb exposure. These data provide evidence that specific

regions of the brainstem involved in auditory integration are affected by developmental Pb exposure via effects on monoamine neurotransmitters. The Pb-induced effects on the monoamine system of the portion of the brainstem involved in auditory function provide possible mechanistic explanation for the epidemiologic and toxicological evidence for Pb-associated decrements in auditory processing (Section 4.3.6.1).

Dopamine

The 2006 Pb AQCD (U.S. EPA, 2006b) detailed evidence for Pb-related decreased dopaminergic cell activity in the substantia nigra and ventral segmental areas. Earlier studies with postnatal or adult Pb exposure reported changes in DA metabolism, as indicated by changes in DA and DOPAC, a DA metabolite. Expanding upon these findings, a recent study assessed DA metabolism in various brain regions of year-old male C57BL/6 mice after gestational plus lactational Pb exposure (Leasure et al., 2008). Both 27 and 109 ppm Pb acetate induced significant elevations in DOPAC concentration and the DOPAC to DA ratio in the forebrain. In the forebrain, DA was significantly decreased with 27 ppm Pb and significantly elevated with 109 ppm Pb compared to controls. In the striatum, DOPAC was significantly elevated with both doses, but DA was significantly elevated only with the higher dose. The striatum ratio of DOPAC to DA was not significantly different from that in controls. These recent data expand upon the monoamine literature base which indicates that Pb exposure of rats during the gestational/lactational, lactational, or postweaning period producing blood Pb levels 9-34 µg/dL increases sensitivity of the dopamine receptors (D2 and D3) (Gedeon et al., 2001; Cory-Slechta et al., 1992), produces higher DA levels (Devi et al., 2005; Leret et al., 2002), and enhances catecholamine neurotransmission in the cerebral cortex, cerebellum, and hippocampus (Devi et al., 2005).

The interaction of DA and the NO system in the striatum was studied after prenatal Pb exposure (Nowak et al., 2008). Blood Pb levels were not reported in this study, but similarly treated Wistar rat pups had blood Pb levels at parturition in range of 50-100 µg/dL (Grant et al., 1980). 7-nitroinidazole (7-NI), a selective inhibitor of nNOS, enhanced amphetamine-evoked DA release in the rat striatum (Nowak et al., 2008). Prenatal Pb exposure attenuated the facilitatory effect of 7-NI on DA release in the striatum. This interaction was ROS-independent; using spin trap measurements, investigators found no significant concentration changes in hydroxyl radical with Pb exposure (Nowak et al., 2008). Thus, the neuronal NO system appears to be involved in certain aspects of Pb-related dopaminergic changes.

In various animal models, the loss of retinal DA, dopamine turnover (DOPAC:DA ratio), or Zn was associated with Pb-induced abnormal rod-mediated scotopic ERGs. These

effects may explain observations of Pb-associated subnormal or supernormal ERGs observed in animals and children (Rothenberg et al., 2002b; Lilienthal et al., 1994; Lilienthal et al., 1988; Alexander and Fishman, 1984) (Section 4.3.6.2), although the biological relevance of the variable effects of Pb exposure on subnormal versus supernormal ERGs is not clear.

NMDA Receptors

The glutamate receptor, NMDAR, has been shown to contribute to synaptic plasticity, and Pb exposure at various developmental stages has been shown to contribute to aberrations in LTP or LTD in the hippocampus via reduced NMDA current, among other mechanisms (Liu et al., 2004). The 2006 Pb AQCD (U.S. EPA, 2006b) indicated that Pb attenuated the stimulation of glutamate release, which in turn, affected LTP. Further, the effects of Pb exposure on decreasing the magnitude of LTP and increasing the threshold of the LTP in the hippocampus were found to be biphasic or nonlinear. NMDAR subtypes have been shown to be significantly decreased with developmental Pb exposure (Guilarte and McGlothan, 1998). Recent evidence indicated Pb-related decreases in the gene expression and protein level of NMDAR subunits NR1, NR2A, and NR2B in weanling male rats exposed to 4×10^4 µg/dL Pb acetate in drinking water for 8 weeks. Several of these responses were attenuated with methionine-choline co-exposure (Fan et al., 2010). Other recent mechanistic studies found that pretreatment of primary fetal brain neuronal rat cultures with glutamic acid, a NMDAR agonist, reversed Pb-induced reductions in NMDAR subunits (Xu and Rajanna, 2006) whereas pretreatment with the NMDA antagonist MK-801 exacerbated Pb-induced NMDAR deficits (Xu and Rajanna, 2006). Further strengthening the link among Pb exposure, NMDAR function, and learning, Guilarte et al. (2003) demonstrated that rats exposed to 1,500 ppm Pb during gestation and lactation then reared in isolation, had reduced expression of hippocampal NR1, reduced induction of BDNF mRNA, and learning impairment. These effects were attenuated in Pb-exposed rats reared in an enriched environment with toys.

Other Glutamate Receptors

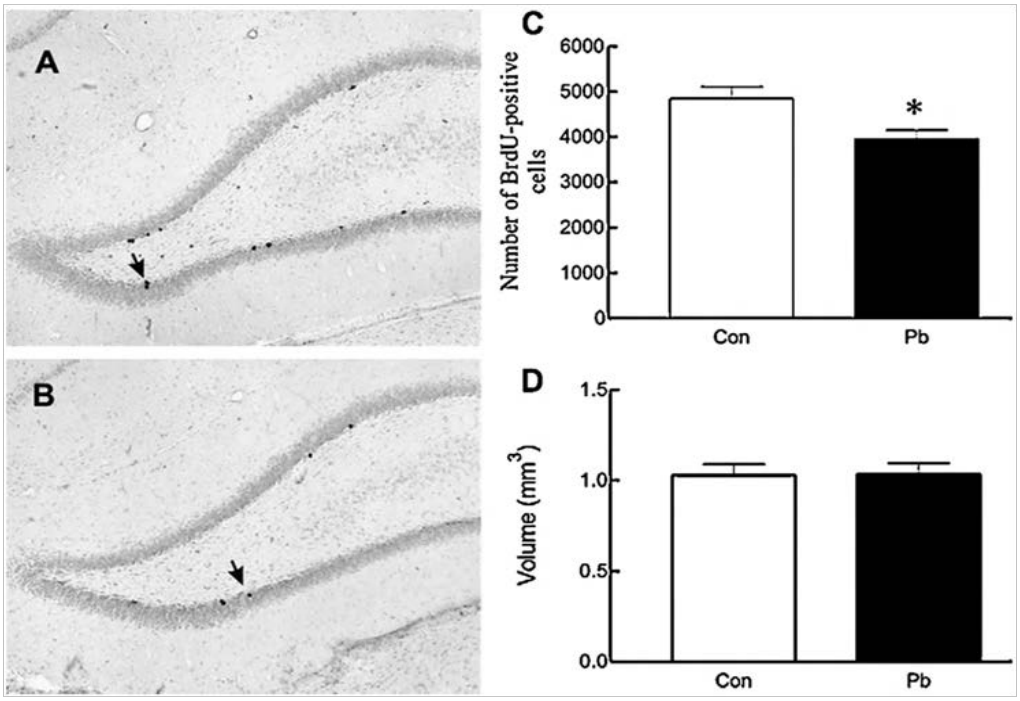
The metabotropic glutamate receptor (mGluR) is another well-recognized target of Pb toxicity. In vitro (GD18 fetal rat hippocampal neurons, 0.01, 100, 1 µM Pb chloride in culture media) and in vivo experiments (gestational-lactational Pb acetate exposure; 500, 2,000, 5,000 ppm in dam drinking water, with respective weanling rat blood Pb levels of 18, 57, 186 µg/dL) showed that Pb exposure decreased mGluR5 mRNA and protein in a concentration-dependent manner (Xu et al., 2009c). Recent evidence indicates a role for

mGluR5 in synaptic plasticity, LTP, and LTD; thus, the Pb-related attenuation of mGlu5 expression may represent a mechanism by which Pb impairs learning and memory.

4.3.10.9 Neurogenesis

Studies continued to show that Pb exposure decreases neurogenesis (i.e., proliferation of neuronal cells) in the hippocampus, which is important in LTP, spatial learning, neuronal outgrowth, and possibly effects related to schizophrenia pathophysiology. Coherence for these findings is provided by evidence for Pb-induced decreases in NMDAR, which mediates the integration of new neurons into existing neuronal pathways in the adult hippocampal DG. Earlier work by Schneider et al. (2005) showed that postnatal Pb exposure (PND25 to PND50 or PND55, 1,500 ppm Pb acetate in chow, resulting in blood Pb level of 20 µg/dL) of male Lewis rats induced significant decrements in BrdU incorporation (an indicator of DNA replication) at PND50-PND55.

Recent publications affirm this previous finding with different sex of animals, dosing and exposure time windows. Postnatal Pb exposure of Wistar rat pups from PND1 to PND30 (2,000 ppm Pb acetate, resulting in blood Pb levels of 34 and 6.5 µg/dL at PND21 and PND80, respectively) induced a statistically significant decrement in the number of new cells (BrdU positive cells) in the DG at PND80 (Fox et al., 2010) (Figure 4-13).



Note: Light micrograph pictures of BrdU positive cells (proliferating cells undergoing DNA replication) shown as black dots, show more proliferating cells in control (A) than Pb-exposed rats (B). Counts of BrdU positive cells (C) but not Volume of hippocampus dentate gyrus (D), are lower in Pb-exposed rats (black bars) than control (white bars) rats. *p <0.05 vs. control. Rats were exposed to 2,000 ppm Pb acetate postnatal day 1-30 (blood Pb levels 34 µg/dL) and were examined at postnatal day 80.

Source: Reprinted with permission of Elsevier Science, Fox et al. (2010)

Figure 4-13 Neurogenesis (production of new cells) in the rat hippocampal dentate gyrus after early postnatal Pb exposure.

In another study, lifetime Pb exposure beginning in gestation (1,500 ppm Pb acetate in chow from 10 days before mating to PND50 or PND78, resulting in blood Pb levels 26 µg/dL) induced significant decrements in hippocampal granule cell neurogenesis in adult Long-Evans rats (Verina et al., 2007). Also, Pb-exposed animals had significant decreases in brain volume in the stratum oriens (SO) region of the hippocampus, specifically in the mossy fiber terminals of the SO. Pb-exposed animals also showed a significant decrease in the length-density of immature or newly-formed neurons in the outer portion of the DG. These findings show that Pb exposure at doses relevant to this ISA induced significant decreases in adult hippocampus granule cell neurogenesis and morphology, potentially providing mechanistic explanations for Pb-induced neuronal aberrations and impairments in downstream effects such as learning and memory. Exposure of zebrafish embryos to Pb (50–700 µM Pb acetate in embryo medium from 0 to 6 days post hatch) caused significant apoptosis of brain cells (increased TUNEL

positive brain cells) and decreased brain levels of some (gfap and huC) but not all (crestin and neurogenin1) genes involved in neurogenesis (Dou and Zhang, 2011).

4.3.10.10 Neurite Outgrowth

As described in the 2006 Pb AQCD (U.S. EPA, 2006b), Pb was shown to decrease neurite outgrowth in vitro and mediate such effects via protein kinase mediated pathways (MAPK/ERK); earlier work had documented decreased primary DA neuron outgrowth with 0.001 µM Pb exposure in vitro (Schneider et al., 2003). A recent study showed that gestational exposure of female Wistar rats to 500-4,000 µM Pb chloride (resulting in offspring blood Pb levels up to 12 µg/dL) significantly decreased offspring hippocampal neurite outgrowth and reduced the expression of hippocampal polysialylated neural cell adhesion molecule (PSA-NCAM), NCAM, and sialyltransferase (Hu et al., 2008b). PSA-NCAM is transiently expressed in newly formed neurons during the period of neurite outgrowth from embryogenesis until the early postnatal period and is down-regulated in the brains of adults except in areas known to exhibit synaptic plasticity (Seki and Arai, 1993). NCAM is important for memory formation, plasticity, and synapse formation, and its suppression by gestational Pb exposure may represent a mechanism that mediates Pb-associated impairments in cognitive function.

4.3.10.11 Epigenetics

Many investigators are beginning to show that environmental chemical exposures and air pollution exposure are associated with epigenetic changes in humans (Baccarelli and Bollati, 2009; Pavanello et al., 2009; Tarantini et al., 2009; Bollati et al., 2007). Epigenetic changes involve changes in DNA expression without changes in the DNA sequence, and these changes may be heritable. Epigenetic changes include changes in histone modification, DNA methylation, miRNA, or pathways that affect these processes. Differential epigenetic modification has the potential to contribute to disease by silencing or activating genes in an aberrant manner. For example, a recent study identified differential methylation of a specific locus in monozygotic twins discordant for schizophrenia (Dempster et al., 2011); Pb was not examined in this study.

DNA methyltransferases catalyze the transfer of a methyl group to DNA and are important in epigenetics (i.e., silencing of genes like tumor suppressors) and imprinting. DNA methyltransferase activity was significantly decreased in cortical neurons from aged monkeys at ages 20-23 years after infancy exposure (1.5 mg/kg Pb acetate via diet per day PND1-PND400, blood Pb level 19-26 µg/dL at PND400) and in fetal mouse

brain cells 1 week after 24-hour exposure to 0.1 µM Pb in culture (Wu et al., 2008a). Cerebral cortex tissue was obtained from female monkeys examined in this study for further analysis. Decreases in DNA methyltransferases (Dnmt1, Dnmt3a) were noted in control monkey brains as they aged, and these decreases were exacerbated by infancy Pb exposure (Bihaqi et al., 2011). Another enzyme involved in DNA methylation, methyl CpG binding protein 2, showed a similar trend as the Dnmts. Profiles of the histone modifying proteins increased with age in control animals. These age-related increases were significantly attenuated in Pb-exposed animals. Methyltransferases catalyze biological methylation reactions using cofactor S-adenosyl methionine (SAM) as the methyl donor. In rats, SAM exposure after gestational-lactational Pb exposure (1,500 ppm Pb acetate via drinking water of dams followed by 20-22 days of daily 20 mg/kg BW SAM exposure of offspring) improved hippocampal LTP and Morris water maze performance at PND44-PND54 (Cao et al., 2008). Thus, the improved cognition and synaptic plasticity observed with co-exposure to Pb and the methyl donor SAM suggest that methylation reactions may be involved in Pb-associated effects on cognition.

4.3.10.12 Cholesterol and Lipid Homeostasis

Various pathological conditions are associated with elevated plasma free fatty acids or elevated cholesterol. Adult male rats exposed to Pb acetate (200, 300, or 400 ppm) in their drinking water for 12 weeks had increased cholesterogenesis and phospholipidosis in brain tissue (Ademuyiwa et al., 2009). Pb-induced changes in brain cholesterol showed an inverse U concentration-response relationship, with the largest increase in brain cholesterol observed with 200 ppm Pb followed by 300 ppm Pb. Animals exposed to 400 ppm Pb did not have significant changes in brain cholesterol. In a separate study, Pb treatment (single dose 100 µmol/kg, i.v.) was shown to depress the activity of cholesterol-7-a-hydroxylase, an enzyme involved in biosynthesis of bile acid, which mediates elimination of cholesterol from the body (Kojima et al., 2005). In Ademuyiwa et al. (2009), Pb exposure significantly increased brain triglycerides by 83% at 300 ppm and by 108% at 400 ppm. At 200 ppm, Pb exposure induced a statistically nonsignificant decrease in brain triglycerides. All three Pb concentrations induced significantly increased brain phospholipids. Interestingly, plasma free fatty acids were significantly elevated in a concentration-dependent manner; plasma triglycerides and cholesterol were unaffected by Pb exposure. The molar ratio of brain cholesterol to phospholipids, an indicator of membrane fluidity, was significantly increased at 200 and 300 ppm Pb exposure, indicating increased membrane fluidity. Brain Pb in all dose groups was below the limit of detection (0.1 ppm). Blood Pb levels for the 0; 200; 300; and 400 ppm Pb exposures were 7; 41; 61; and 39 µg/dL, respectively; higher than those relevant to this

ISA. In summary, a recent study found that adult 12-week Pb exposure significantly increased brain cholesterol, triglycerides, and phospholipids as well as significantly increased plasma free fatty acids in rats. The increase in cholesterol was more prominent at the lower 200 ppm Pb dose. The impacts of these Pb-related changes in phospholipidosis and cholesterogenesis in the brain on downstream nervous system effects are not well characterized.

4.3.11 Lifestages and Time Periods of Pb Exposure and Neurodevelopmental Effects

Environmental exposures during critical lifestages can affect key physiological systems that orchestrate plasticity (Feinberg, 2007). Exposures during the prenatal and/or early postnatal period may be especially detrimental for neurodevelopmental effects because of active neuronal growth and/or synaptogenesis/pruning structure that occur during these periods (Rice and Barone, 2000; Nolte, 1993). However, brain development has been shown to continue throughout adolescence. MRI studies of the Child Psychiatry Branch cohort of children and adults ages 3-30 years have shown peak total cerebral volume at age 10.5 and 14.5 years in females and males, respectively (Giedd et al., 2009; Lenroot and Giedd, 2006). The volume of the cerebellum was found to peak two years later. Lateral ventricular volume and white and gray matter volume also were found to increase throughout adolescence. Gray matter volume peaked 1 to 3 years earlier in females than males. These observations that brain development is active throughout childhood and in adolescence indicate the potential for neurodevelopment to be altered later in childhood.

Epidemiologic studies consistently show that blood Pb levels measured during various lifestages and time periods, including the prenatal period, early childhood, later childhood, and averaged over multiple years, are associated with cognitive function decrements and increases in externalizing and internalizing behaviors. These observations of Pb-associated elevated risk of neurodevelopmental decrements in children are well supported by findings in animals that prenatal and/or early postnatal or lifetime Pb exposure alters brain development via changes in synaptic architecture (Section 4.3.10.4) and neuronal outgrowth (Section 4.3.10.10) and leads to impairments in memory and learning (Section 4.3.2.3) and increases in impulsivity (Section 4.3.3.1). In monkeys, Pb exposures during multiple lifestages and time periods, including lifetime, infancy, or juvenile to adulthood, resulted in impaired cognitive function, although not on all tests (Rice, 1992b; Rice and Gilbert, 1990a; Rice, 1990; Rice and Karpinski, 1988). On one test of executive function in the same monkeys at ages 5-6 years, impairments were found with lifetime Pb exposure starting from birth or starting at age 400 days but not infancy-only exposure (Rice and Gilbert, 1990b). The observations indicate that

gestational or infancy Pb exposures are not necessary to induce cognitive function decrements in juvenile animals.

Unlike other organ systems, the unidirectional nature of CNS development limits the capability of the developing brain to compensate for cell loss, and environmentally-induced cell death can result in a permanent reduction in cell numbers (Bayer, 1989). Hence, when normal development is altered, the early effects have the potential to persist into adult life even in the absence of concurrent exposure, magnifying the potential public health impact. Some epidemiologic evidence indicates associations of earlier childhood blood or tooth Pb levels with cognitive function decrements, attention decrements, and increases in behaviors related to conduct disorders in adolescents or adults (Mazumdar et al., 2011; Fergusson et al., 2008; Wright et al., 2008; Ris et al., 2004; Bellinger et al., 1994a; Stiles and Bellinger, 1993). These epidemiologic studies did not examine adult blood Pb levels, thus the relative influence of adult Pb exposure cannot be ascertained. In the Boston cohort, stronger associations observed for age 2 year blood Pb level than concurrent blood Pb level with FSIQ decrements at ages 57 months and 10 years indicated an effect of earlier rather than later childhood Pb exposures (Bellinger et al., 1992; 1991). The persistence of effects of early exposures is supported by findings of impaired learning in adults monkeys that had infancy-only Pb exposure (Rice, 1992b; Rice and Gilbert, 1990a; Rice, 1990). A few available toxicological studies also found that infancy Pb exposure (but not adult exposure in rats) led to neurodegenerative amyloid plaque formation in the brains of aged rodents and monkeys (Section 4.3.9.1).

With repeated assessments of children (prenatally to later childhood and early adulthood), the prospective cohort studies have provided data to compare the neurodevelopmental effects associated with blood Pb levels measured at different lifestages and time periods. In the collective body of evidence, cognitive function decrements in children have been associated with prenatal, early childhood, childhood average, and concurrent blood Pb levels, without clear indication of a single critical lifestage or duration of Pb exposure related to risk of neurodevelopmental effects in children. The identification of critical developmental periods with regard to risk of neurodevelopmental decrements from Pb exposure has been complicated by the high degree of correlation of the blood Pb levels of children over time and the confounding of age and peak blood Pb levels (Hornung et al., 2009; Lanphear et al., 2005; Dietrich et al., 1993a).

As described in detail in the 2006 Pb AQCD (U.S. EPA, 2006b), several studies with varying lengths of follow-up demonstrated associations of prenatal blood Pb levels (maternal and umbilical cord) with decrements in cognitive function throughout childhood and into early adulthood (Section 4.3.2). These findings are consistent with the observations of active CNS development occurring during prenatal development as

described above. Substantial fetal Pb exposure may occur from mobilization of maternal skeletal Pb stores, which may be related to past maternal Pb exposures (Gulson et al., 2003; Hu and Hernandez-Avila, 2002), and the transfer of Pb across the placenta (Section 3.2.2.4). Among 94-211 mother-child pairs in Albany County, NY, maternal pregnancy-cord blood Pb level correlations of 0.53-0.81 were reported, depending on the stage of pregnancy, indicating the influence of maternal blood Pb levels on newborn blood Pb levels (Schell et al., 2003). Depending on the magnitude of child exposure, the contribution of maternal blood Pb on child blood Pb levels appears to diminish rapidly over a period of a few months following birth, after which child blood Pb levels may be influenced mainly by postnatal Pb exposures (Section 3.4.1). Thus, associations of neurodevelopmental outcomes assessed after infancy with postnatal blood Pb levels may reflect effects of postnatal Pb exposures.

In most studies of very young children, ages <2 years, decrements in MDI score were associated with higher prenatal (maternal or cord) and concurrent blood Pb levels (Table 4-14). Among studies that examined blood Pb levels at multiple time periods, some found a larger magnitude decrement in MDI per unit increase in prenatal blood Pb than concurrent blood Pb (Hu et al., 2006; Gomaa et al., 2002) (Table 4-14). The mean was higher for prenatal blood Pb level than concurrent in one study (Hu et al., 2006), whereas another study reported similar means for prenatal and concurrent blood Pb levels (Gomaa et al., 2002). Bellinger et al. (1987) did not report effect estimates for concurrent blood Pb level and only indicated lack of statistically significant association with MDI. In the Yugoslavia cohort, per log increase in blood Pb level, the MDI decrement at age 2 years was larger for concurrent blood Pb than for prenatal cord blood Pb (Wasserman et al., 1992). Concurrent blood Pb levels were higher than prenatal cord blood Pb levels. The collective evidence indicates that both prenatal and postnatal child Pb exposures may contribute to neurodevelopmental effects in children at to age 2 years, with some indication that prenatal Pb exposure has a stronger effect.

Table 4-14 Associations of cognitive function with blood Pb levels measured at various lifestages and time periods in prospective studies.

Study[a]	Study Population and Methodological Details Within studies, effect estimates are presented in order of lifestage or time period of blood Pb measurement, with largest effect estimate in bold[a]	Blood Pb Metrics Analyzed (µg/dL)	Outcome Analyzed	Effect Estimate (95% CI)[b]
Cognitive Assessment at Age 2 Years and Younger				
Bellinger et al. (1987)	182 children followed from birth (1979-1981) to age 2 yr, Boston, MA area. Recruitment from birth hospital. High follow-up participation. Participants had higher cord blood Pb level, SES, HOME score, maternal education and IQ, lower maternal age, were white. Regression model adjusted for maternal age, education, race, IQ, years of smoking, and alcohol drinks/week in 3rd trimester, SES, HOME score, child sex, birth weight, gestational age, birth order.	Prenatal (cord) Mean (SD): 6.6 (3.2) Low: < 3 Medium: 6-7 High: ≥ 10	Overall Bayley MDI among Ages 6, 12, 18, and 24 mo	Score in high cord blood Pb group **vs. Low cord blood: -4.8 (-7.3, -2.3)[c]** **vs. Medium cord blood: -3.8 (-6.3, -1.3)[c]** Concurrent reported to not to be significantly associated with overall MDI, no quantitative data reported.
Dietrich et al. (1986)	245-280 children followed prenatally to age 6 mo, Cincinnati, OH No information on participation rate. Log linear regression model adjusted for birth weight, gestation, sex. Also considered potential confounding by SES, HOME score, prenatal smoking and alcohol use, maternal Fe binding capacity, child sex, race.	Mean (SD) Prenatal (maternal): 8.0 (3.8) Concurrent: 4.5 (2.9)	Bayley MDI Age 6 mo	Per log increase in blood Pb: Prenatal: -15 (-27, -3) Concurrent: -15 (-25, -5)
Hu et al. (2006)	83-146 children born 1997-1999 followed prenatally to age 2 yr, Mexico City, Mexico Recruitment from prenatal clinic. Moderate follow-up participation. Eligible similar to non-eligible. Log linear regression model adjusted for maternal age and IQ, child sex, current weight, height-for-age Z score, and concurrent blood Pb (in models examining prenatal blood Pb). Considered potential confounding by other unspecified factors.	Mean (SD) Prenatal (maternal 1st trimester): 7.1 (5.1) Prenatal avg NR Earlier childhood at 12 mo: 5.2 (3.4) Concurrent: 4.8 (3.7)	Bayley MDI Age 24 mo	Per log increase in blood Pb: **Prenatal 1st trimester: -4.1 (-8.1, -0.17)** Prenatal avg: -3.5 (-7.7, 0.63) Age 12 month: -2.4 (-6.2, 1.5) Concurrent: -1.0 (-3.9, 1.9)

Table 4-14 (Continued): Associations of cognitive function with blood Pb levels measured at various lifestages and time periods in prospective studies.

Study[a]	Study Population and Methodological Details Within studies, effect estimates are presented in order of lifestage or time period of blood Pb measurement, with largest effect estimate in bold[a]	Blood Pb Metrics Analyzed (µg/dL)	Outcome Analyzed	Effect Estimate (95% CI)[b]
Gomaa et al. (2002)	161-197 children followed from birth to age 2 yr, Mexico City, Mexico Recruitment from birth hospital. Moderate participation but high retention, no selective attrition. Log linear regression model adjusted for maternal IQ, age, parents in home, breastfeeding duration, parental education, child hospitalization status, and sex. Did not consider potential confounding by parental caregiving quality.	Mean (SD) Prenatal (cord): 6.7 (3.4) Concurrent: 8.4 (4.6)	Bayley MDI Age 24 mo	Per log increase in blood Pb: **Prenatal: -4.9 (-9.0, -0.89)** Concurrent: -0.09 (-0.58, 0.40)
Wasserman et al. (1992)	392 children followed prenatally to age 2 yr, K. Mitrovica, Pristina, Yugoslavia. 53% live near Pb sources. High follow-up participation, no selective attrition. Log linear regression model adjusted for sex, birth order, birth weight, ethnic group, HOME score, maternal education, age, and IQ.	Mean (SD) Prenatal (cord): 14 (10) Concurrent: 35 K. Mitrovica, 8.5 Pristina	Bayley MDI Age 24 mo	Per log increase in blood Pb: Prenatal: -3.5 (-8.6, 1.7) **Concurrent: -5.3 (-10, -0.53)**
Ernhart et al. (1988; 1987)	146-165 children, followed prenatally to age 2 yr, Cleveland, OH **Prospective.** Recruitment at birth hospital. High follow-up participation. More white, higher IQ, nonalcohol using mothers not followed. 50% born to alcoholic mothers. Linear regression adjusted for age, race, sex, birth order, birth weight, parental education, maternal IQ, Authoritarian Family Ideology, HOME score.	Mean (SD) Prenatal cord: 6.0 (1.8) 6 mo: 10 (3.3) Concurrent: 17 (6.5)	Bayley MDI Age 2 yr	Variance estimates: Prenatal: 0.0003, t = -0.21[d] Age 6 mo: 0.00, p = 0.95[d] Concurrent: 0.00, p = 0.95[d]
Cognitive Assessment at Age 3 Years and Older				
Canfield et al. (2003a)	171-172 children born 1994-1995 followed from age 6 mo to 5 yr, Rochester, NY Recruitment from study of dust control. 73% nonwhite. Moderate follow-up participation but no selective attrition. Linear regression model adjusted for child sex, Fe status, birth weight, maternal race, education, IQ, income, and prenatal smoking status, HOME score.	Mean (SD) Infancy avg (6-24 mo): 7.0 (3.8) Peak: 11 (7.1) Concurrent: 5.8 (4.1) Lifetime (to age 5 yr) avg: 7.4 (4.3)	FSIQ Stanford-Binet Age 5 yr	Per 1 µg/dL increase in blood Pb: Infancy avg: -0.53 (-0.93, -0.13) Peak: -0.26, (-0.47, -0.05) **Concurrent: -0.61 (-0.99, -0.24)** **Lifetime avg: -0.57 (-0.93, -0.20)**

Table 4-14 (Continued): Associations of cognitive function with blood Pb levels measured at various lifestages and time periods in prospective studies.

Study[a]	Study Population and Methodological Details Within studies, effect estimates are presented in order of lifestage or time period of blood Pb measurement, with largest effect estimate in bold[a]	Blood Pb Metrics Analyzed (µg/dL)	Outcome Analyzed	Effect Estimate (95% CI)[b]
Wasserman et al. (1994)	332 children followed prenatally to ages 3-4 yr (1991), K. Mitrovica, Pristina, Yugoslavia Moderate follow-up participation. More participants were male, from K. Mitrovica, Albanian, and had lower maternal IQ and education and HOME score. Log linear regression model adjusted for HOME score, maternal age, IQ, and education, language, sibship size, birth weight, child sex.	Mean (SD) Prenatal (cord): 14 (11) Geometric means Concurrent K. Mitrovica: 40 Pristina: 9.6	FSIQ McCarthy GCI Age 4 yr	Per log increase in blood Pb: Prenatal cord: -7.1 (-12, -3.1) **Age 2 yr: -10.4 (-15, -5.7)** Concurrent: -9.4 (-14, -4.6)
Wasserman et al. (2000a)	390 children followed prenatally (1985-1896) to age 7 yr, K. Mitrovica, Pristina, Yugoslavia Moderate follow-up participation. More participants were from K. Mitrovica, Albanian, with lower maternal IQ and education and HOME score. Regression model adjusted for HOME score, maternal age, education, IQ, and # live births, child sex, birthweight, ethnicity, age.	Mean of average during period Prenatal:10 Age 0-2: 13 Age 2-3: 19 Age 2-4: 19 Age 2-5: 19 Age 2-7: 17	FSIQ McCarthy GCI, ages 3-4 yr WPPSI, age 5 yr WISC-III, age 7 yr	Prenatal: -6.1 (-8.7, -3.4) per log increase in blood Pb Postnatal blood Pb analyzed as categorical variable No increase prenatal to postnatal: Reference ≥50% increase in postnatal only from birth to age 2 yr: -1.8 (-3.5, -0.05)[c] ≥50% increase in postnatal from birth to age 2 yr and from age 2 yr onward: -2.7 (-4.9, -0.52)[c]
Bellinger et al. (1992)	148 children followed from birth (1979-1981) to age 10 yr, Boston, MA area Moderate follow-up participation. Participants had higher SES and HOME scores. Linear regression model adjusted for HOME score (age 10 and 5 yr), maternal race, age, IQ, and marital status, SES, child sex, birth order, and stress. Plus: # residence changes (2 yr), family stress, birth weight, # daycare situations to 5 yr (10 yr). Also considered potential confounding by psychiatric factors, child serum ferritin levels.	Mean (SD) Age 6 mo: 6.7 (7.0) Earlier childhood Age 2 yr: 6.5 (4.9) Concurrent: 2.9 (2.4)	FSIQ WISC-Revised Age 10 yr	Per 1 µg/dL increase in blood Pb: Age 6 mo: -0.13 (-0.42, 0.16) **Age 2 yr: -0.58 (-0.99, -0.17)** Concurrent -0.46 (-1.5, 0.56)

Table 4-14 (Continued): Associations of cognitive function with blood Pb levels measured at various lifestages and time periods in prospective studies.

Study[a]	Study Population and Methodological Details Within studies, effect estimates are presented in order of lifestage or time period of blood Pb measurement, with largest effect estimate in bold[a]	Blood Pb Metrics Analyzed (µg/dL)	Outcome Analyzed	Effect Estimate (95% CI)[b]
Dietrich et al. (1993b)	199-231 children followed from birth (1979-1985) to age 6.5 yr, Cincinnati, OH Recruitment at prenatal clinic. High follow-up participation. Participants had slightly higher age 1 yr blood Pb levels. Primarily African-American. Linear regression model adjusted for HOME score, child birth weight, birth length, and sex, maternal IQ and prenatal smoking. Also considered potential confounding by perinatal complications, prenatal maternal substance abuse, nutritional status.	Mean (SD) Prenatal (maternal) 8.3 (3.7) Age 4-5 yr avg 11.8 (6.3) Concurrent: NR Lifetime (to age 6.5 yr) avg: NR	FSIQ WISC-Revised Age 6.5 yr	Per 1 µg/dL increase in blood Pb: Prenatal: 0.15 (-0.26, 0.56) **Concurrent: -0.33 (-0.60, -0.06)** Lifetime avg: -0.13 (-0.35, 0.09)
Baghurst et al. (1992)	494 children followed from birth (1979-1982) to ages 7-8 yr, Port Pirie, Australia Moderate follow-up participation. Participants had higher SES, more breastfeeding, less maternal smoking. Log linear regression model adjusted for sex, birth weight, birth order, feeding method, breastfeeding duration, parental education and smoking status, maternal age and IQ, SES, HOME score, parents living together.	Means of 2nd quartile Prenatal (cord): 7.4 Age 0-2 yr: 17 Lifetime (to age 7 yr) avg: 16	FSIQ WISC-Revised Age 7-8 yr	Per log increase in blood Pb: Prenatal: 0.6 (-2.1, 3.3) **Age 0-2 yr: -4.6 (-8.7, -0.48)** Lifetime avg: -3.7 (-8.6, 1.2)
Schnaas et al. (2006)	136-150 children followed from birth (1987-1992) to age 10 yr, Mexico City, Mexico. Recruitment at prenatal clinic. Low follow-up participation. Participants had higher SES, FSIQ, higher blood Pb level before age 5 yr, lower at older ages. Log linear mixed effects model adjusted for SES, maternal IQ, child sex, birth weight, & postnatal blood Pb, age of 1st FSIQ test, random slope for subject. Also considered potential confounding by HOME score. Covariates assessed in pregnancy or within age 6 mo.	Geometric Mean (5th-95th) Prenatal (maternal 28-36 week): 7.8 (2.5-25) Age 5 yr: 9.3 (3.8-18) Age 6-10 yr avg: 6.2 (range = 2.2-19)	FSIQ WISC-Revised Ages 6-10 yr	Per log increase in blood Pb: **Prenatal: -4.0 (-6.4, -1.7)** Age 5 yr: -0.32 (-4.3, 3.4) Age 6-10 yr avg: -2.5 (-4.1, -0.81)

Table 4-14 (Continued): Associations of cognitive function with blood Pb levels measured at various lifestages and time periods in prospective studies.

Study[a]	Study Population and Methodological Details Within studies, effect estimates are presented in order of lifestage or time period of blood Pb measurement, with largest effect estimate in bold[a]	Blood Pb Metrics Analyzed (µg/dL)	Outcome Analyzed	Effect Estimate (95% CI)[b]
Ris et al. (2004)	195 children followed prenatally (1979-1985) to age 15-17 yr, Cincinnati, OH **Prospective**. Recruitment at prenatal clinic. High follow-up participation, no selective attrition. Mostly African-American. Linear regression model adjusted for SES, maternal IQ, HOME, adolescent marijuana use, obstetrical complications. Also considered potential confounding by birth outcomes, maternal age, maternal prenatal smoking, alcohol, marijuana, and narcotics use, # previous abortions, stillbirths, gravidity, parity, public assistance, child sex, age, health and Fe status, caregiver education.	Prenatal, Earlier childhood (age 6.5 yr), Earlier childhood avg (age 3-78 mo): NR	Learning/IQ factor score WISC-III, WRAT-3 indices Ages 15-17 yr	Per 1 µg/dL increase in blood Pb: **Prenatal: -0.08 (-0.18, 0.03)** **Age 6.5 yr: -0.08 (-0.17, 0.00)** Age 3-78 mo avg: -0.03 (-0.09, 0.02)
Lanphear et al. (2005)	1,333 children pooled from Boston, Cincinnati, Cleveland, Mexico City, Port Pirie, Rochester, and Yugoslavia cohorts. Uniform analytic method applied to cohorts from diverse locations and demographic characteristics. Blood Pb levels and FSIQ measured at different ages. Several sensitivity analyses to examine heterogeneity of results by cohort, confounding, and model specification. Log linear regression model adjusted for HOME score, birth weight, maternal IQ and education. Also considered potential confounding by child sex, birth order, marital status, maternal age, prenatal smoking status and alcohol use.	Median (5th-95th) Early childhood (mean ages 6-24 mo): 12.7 (4.0-35) Peak: 18 (6.2-47) Lifetime avg (to ages 4.8-10 yr): 12 (4.1-35) Concurrent: 9.7 (2.5-33)	FSIQ Various tests Ages 4.8-10 yr	Per log increase in blood Pb: Early childhood: -2.0 (-3.3, -0.81) **Peak: -2.9 (-4.1, -1.6)** **Concurrent: -3.0 (-4.3, -1.8)** Lifetime avg: -2.7 (-3.7, -1.7)

Table 4-14 (Continued): Associations of cognitive function with blood Pb levels measured at various lifestages and time periods in prospective studies.

Study[a]	Study Population and Methodological Details Within studies, effect estimates are presented in order of lifestage or time period of blood Pb measurement, with largest effect estimate in bold[a]	Blood Pb Metrics Analyzed (µg/dL)	Outcome Analyzed	Effect Estimate (95% CI)[b]
Pocock et al. (1994)	Meta-analysis of 5 prospective studies (1,260 children) from Port Pirie and Sydney, Australia, Cincinnati, Cleveland, Boston Meta-analysis of combining covariate-adjusted effect estimates from individual studies.	Earlier childhood (2 yr) range in means: 6.8-21 Around birth, Postnatal: NR	FSIQ Various tests Ages 5-10 yr	Per doubling of blood Pb from 10-20 µg/dL: Around birth: 0.18 (-1.0, 1.4) Age 2 yr: -1.9 (-2.8, -0.85) Postnatal mean: -0.88 (-2.0, 0.26)

MDI = Mental Development Index, FSIQ = full-scale IQ, NR = Not reported, McCarthy GCI = McCarthy General Cognitive Index, WPPSI = Wechsler Preschool and Primary Scale of Intelligence, WISC = Wechsler Intelligence Scale for Children

[a]Results are presented first for MDI in children up to age 2 years, then for FSIQ in children ages 3 years and older. Within studies, effect estimates are presented in order of increasing lifestage or time period of blood Pb measurement, with the largest effect estimate in bold. After age of assessment, studies are grouped by strength of study design, representativeness of the study population characteristics and blood Pb levels examined, and extent of consideration for potential confounding. There is not necessarily a continuum of decreasing strength across studies.

[b]Effect estimates are standardized to a 1 µg/dL or log increase in blood Pb level in analyses of blood Pb as a continuous variable.

[c]Effect estimates represent comparisons between children in different categories of blood Pb level, with children in the lower blood Pb category serving as the reference group.

[d]Sufficient data were not available to calculate 95% CI.

Prenatal and early postnatal (age 6 month) blood Pb levels also were associated with cognitive function in studies that included school-aged children (ages 5-17 years) (Table 4-14). However, most of these studies also found cognitive function decrements in association with postnatal blood Pb levels, and results did not identify an individual critical postnatal time period of blood Pb measurement associated with cognitive function decrements. In the Yugoslavia cohort, the FSIQ decrement per unit increase in blood Pb level was larger for prenatal maternal than concurrent blood Pb when children were examined at ages 3-7 years (Wasserman et al., 2000a), but larger for concurrent than prenatal maternal or cord blood Pb when children were examined at age 4 years (Wasserman et al., 1994). In the Cincinnati cohort, the association with FSIQ was stronger for concurrent than for prenatal blood Pb levels at age 6.5 years (Dietrich et al., 1993b), but in adolescents ages 15-17 years, increases in both prenatal and earlier childhood (age 6.5 years) blood Pb levels were associated with decrements in a learning/memory composite score (Ris et al., 2004). In the Boston and Port Pirie cohorts, increases in age 2 year or 0-2 year average blood Pb levels were associated with larger FSIQ decrements at ages 10 and 7 years than were concurrent or lifetime average blood Pb levels, respectively (Baghurst et al., 1992; Bellinger et al., 1992). However, in the Port Pirie cohort, were was an association found for lifetime average (to age 7 years) blood Pb level. Among children ages 6-10 years in Mexico City, per unit increase, prenatal blood Pb level was associated with a larger FSIQ decrement than were blood Pb levels measured at each age from 1 to 5 years or averaged from age 6 to 10 years (Schnaas et al., 2006). In contrast, results from the Rochester cohort at age 5 years indicated that the FSIQ decrement per unit increase in blood Pb level was larger for lifetime average and concurrent blood Pb than peak blood Pb (Canfield et al., 2003a). Collectively, the epidemiologic findings indicate that blood Pb levels measured at various postnatal time periods, early childhood (e.g., age 2 year), lifetime average (e.g., to 7 years), later childhood (e.g., age 6-10 year average), and concurrent blood Pb levels are associated with decrements in cognitive function when assessed in populations that include school-aged children ages 5-17 years.

Consistent with individual studies, analyses combining studies pointed to associations of FSIQ in school-aged children with blood Pb levels measured at various lifestages and time periods. The analysis pooling data from seven prospective studies found that increases in early childhood (age 6-24 month average), peak, concurrent, and lifetime average blood Pb levels were associated with decreases in FSIQ in children ages 4.8-10 years (Table 4-14). Investigators reported that the model with concurrent blood Pb level explained the largest proportion of variance in FSIQ (R^2) (Lanphear et al., 2005). In a meta-analysis of results from five prospective studies (Pocock et al., 1994), the decrease in FSIQ per unit increase in blood Pb level was larger for peak (around age 2 years) blood Pb level than blood Pb level measured around birth or after age 2 years. Deciduous

tooth Pb levels have been associated with decrements in cognitive function, decrements in attention, as well as increases in impulsivity and hyperactivity in children and young adults (Table 4-8, Table 4-9, and Table 4-11). These results that indicate cumulative Pb exposure over several years may contribute to neurodevelopmental effects in children. In school-aged children, concurrent blood Pb levels reflect recent exposures and past Pb exposures that are mobilized from bone remodeling to blood. Thus, associations with concurrent blood Pb levels also may reflect an effect of cumulative past and more recent Pb exposures.

Studies conducted in the Cincinnati cohort examined diverse nervous system effects and found that prenatal or neonatal blood Pb levels were associated with poorer auditory processing in children ages 5 years (Dietrich et al., 1992) and higher parental ratings of delinquent behavior in adolescents ages 15-17 years (Dietrich et al., 2001) but inconsistently with motor function decrements at ages 4-10 years (Bhattacharya et al., 2006; Bhattacharya et al., 1995; Dietrich et al., 1993a) and not with cognitive function in children at age 6 years (Dietrich et al., 1993b). These findings suggest that the critical timing of Pb exposure may vary among nervous system endpoints.

Some studies have aimed to improve the characterization of important lifestages and time periods of Pb exposure by examining children in whom blood Pb levels are not strongly correlated over time (i.e., children whose blood Pb level ranking changed over time) (Hornung et al., 2009; Schnaas et al., 2006; Chen et al., 2005a; Wasserman et al., 2000a; Tong et al., 1998; Bellinger et al., 1990). Some calculated specific blood Pb metrics that reflected the change over time (i.e., ratio of blood Pb measured at different times, amount of change over time), and collectively most results indicated FSIQ decrements in association with higher concurrent blood Pb levels but did not conclusively demonstrate stronger findings for early or concurrent blood Pb levels (Table 4-15).

Schnaas et al. (2006) did not calculate a blood Pb level metric to reflect the change over time but found a weak correlation between maternal blood Pb levels at 28-36 weeks of pregnancy and repeated measures of child blood Pb level between ages 1 and 10 years (Pearson $r \leq 0.23$). In this study, children in Mexico City were followed prenatally to age 10 years. In models analyzing blood Pb levels at different ages individually or together in one mixed effects model that included prenatal and multiple postnatal blood Pb measures, the decrement in FSIQ per unit increase in blood Pb level was largest for maternal 28-36 week blood Pb level (Table 4-14). In the model that included blood Pb level at multiple ages, analysis of variance inflation factors indicated a lack of collinearity among the serial blood Pb measures

Table 4-15 Comparisons of blood Pb-cognitive function associations in groups of children with different temporal trends in blood Pb levels.

Study	Study Population and Methodological Details[a]	Blood Pb Analyzed (µg/dL)	Outcome Analyzed	Effect Estimate (95% CI)
Hornung et al. (2009)	397 children (born 1979-1995) followed from age 2 to 6 yr, Rochester, NY and Cincinnati, OH. Moderate follow-up participation, representative of full cohorts. Regression model adjusted for city, HOME score, birth weight, maternal IQ and education, log childhood average blood Pb level.	Geometric mean (5th-95th): Early childhood age 2 yr: 8.9 (3.0-24) Concurrent: 6.0 (1.9-18) 6.3% with higher age 6 yr than age 2 yr blood Pb level	FSIQ WPPSI and WISC-R Age 6 yr	Difference in FSIQ at age 6 yr per 1 unit increase in age 6:2 year blood Pb ratio: -7.0 (-10, -4.0) Estimates a 7-point lower FSIQ in children with 50% higher blood Pb level at age 6 than 2 years (ratio = 1.5) compared to children with a 50% lower blood Pb level at age 6 than 2 years (ratio = 0.5)
Bellinger et al. (1990)	170 children followed prenatally to age 57 mo, Boston, MA area High follow-up participation, no comparison with nonparticipants. Log regression adjusted for HOME score, SES, maternal IQ and age, sex, ethnicity.	High prenatal (cord): >10 Concurrent Low: <3 Medium: 3-10 High: >10	Change in z-score McCarthy GCI at age 57 mo MDI at 24 mo	High prenatal/Low concurrent 0.42 (-0.15, 0.99) High prenatal/Medium concurrent 0.15 (-0.14, 0.44) High prenatal/High concurrent -0.15 (-0.56, 0.26)
Wasserman et al. (2000a)	390 children followed prenatally (1985-1986) to age 7 yr, K. Mitrovica, Pristina, Yugoslavia Moderate follow-up participation. More participants were from K. Mitrovica, Albanian, with lower maternal IQ and education and HOME score. Regression model adjusted for HOME score, maternal age, education, IQ, and # live births, child sex, birthweight, ethnicity, age.	Mean of average during period Prenatal:10 Age 0-2: 13 Age 2-3: 19 Age 2-4: 19 Age 2-5: 19 Age 2-7: 17	FSIQ McCarthy GCI, ages 3-4 yr WPPSI, age 5 yr WISC-III, age 7 yr	No change from prenatal to postnatal: Reference ≥50% increase prenatal to postnatal only birth to age 2 yr: -1.8 (-3.5, -0.05) ≥50% increase prenatal to postnatal from birth to age 2 yr and from age 2 yr onward: -2.7 (-4.9, -0.52)

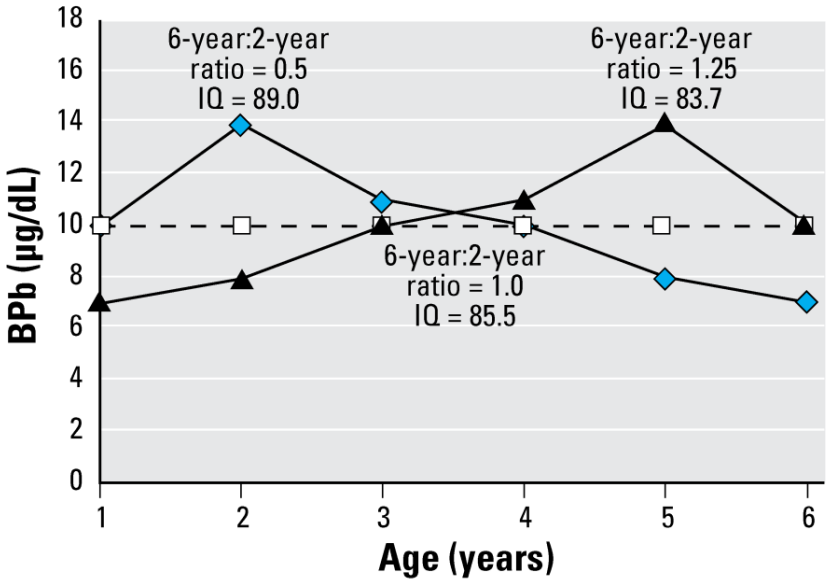
Table 4-15 (Continued): Comparisons of blood Pb-cognitive function associations in groups of children with different temporal trends in blood Pb levels.

Study	Study Population and Methodological Details[a]	Blood Pb Analyzed (µg/dL)	Outcome Analyzed	Effect Estimate (95% CI)
Tong et al. (1998)	326 children followed from birth (1979-1982) to age 11-13 yr, Port Pirie, Australia Moderate follow-up participation. Participants had weaker early blood Pb-cognitive function association. ANOVA to compare mean change in FSIQ among children with different degrees of change in blood Pb across time.	Means Early childhood age 2 yr: 21 Age 11-13 yr: 7.9	Mean change in cognitive function z-scores between ages 2 and 11-13 yr Bayley MDI, age 2 yr WISC-R, ages 11-13 yr	<10.2 µg/dL decline: 0.03 (-0.15, 0.21) 10.2-16.2 µg/dL decline: 0.04 (-0.15, 0.23) >16.2 µg/dL decline: -0.01 (-0.20, 0.18) p = 0.74 for difference among groups
Chen et al. (2005a)	617 children participating in the TLC trial from age 12-33 mo to age 7 yr, Baltimore, MD; Cincinnati, OH; Newark, NJ 77% African-American. 50% given chelation at ages 12-33 mo, blood Pb levels 20-44 µg/dL. High follow-up participation. Regression model adjusted for city, race, sex, age at blood Pb measurement, language, single parent, caregiver IQ, parental education and employment. Considered potential confounding by chelation but not parental caregiving quality.	Mean (SD): Age 2 yr: 26 (5.1) Age 5 yr: 12 (5.2) Age 7 yr: 8.0 (4.0) Low age 2 yr: <24.9 Low age 7 yr: <7.2	WISC-III Age 7 yr	Difference in age 7 FSIQ Low age 2, Low age 7: Reference Low age 2, High age 7: -3.6 (-6.4, -0.7) High age 2, Low age 7: 0 (-2.8, 2.7) High age 2, High age 7: -3.7 (-6.2, -1.3)

WPPSI = Wechsler Preschool and Primary Scale of Intelligence, WISC = Wechsler Intelligence Scale for Children, GCI = General Cognitive Index

[a]Studies are grouped by strength of study design, representativeness of the study population characteristics and blood Pb levels examined, and extent of consideration for potential confounding. There is not necessarily a continuum of decreasing strength across studies.

Combining the Cincinnati and Rochester cohorts (n = 397), Hornung et al. (2009) analyzed the ratio of age 6 blood Pb level to age 2 year blood Pb level to assess the association of FSIQ at age 6 years with a change in blood Pb level over time. Blood Pb levels measured at various ages were highly correlated (r = 0.64-0.96), but investigators found a range of temporal trends in blood Pb levels (range in age 6:2 year blood Pb level: 0.11-4.1, mean: 0.58). This range was analyzed in the model as the predictor. The model estimated that for children with the same average (ages 6 months-6 years) blood Pb level, those with a higher age 6:2 year blood Pb ratio had a lower FSIQ at age 6 years (Table 4-15). Although 6.3% of children had a ratio > 1, representing higher age 6 than age 2 blood Pb level, model results were not dependent on these data points. An example of model results is illustrated in Figure 4-14. The three temporal blood Pb patterns have similar areas under the curve, indicating similar childhood average blood Pb levels. Thus, differences in FSIQ more likely would be attributable to differences in temporal exposure pattern. Among the three blood Pb patterns illustrated, the model estimated the lowest FSIQ for children with an age 6:2 year blood Pb ratio of 1.25 or children who had a 25% increase in blood Pb level from 2 to 6 years of age (Figure 4-14).



Note: FSIQ was estimated for different patterns of blood Pb level changes over time: 1) peak at age 2 years, i.e., age 6:2 year ratio = 0.5 (blue diamonds); 2) peak at 5 years, i.e., age 6:2 year ratio = 1.25 (black triangles); and 3) constant blood Pb level, i.e., age 6:2 year ratio = 1 (white squares). The 3 patterns have a similar cumulative blood Pb level (10 µg/dL) as indicated the areas under the curve. Among the 3 patterns, the lowest FSIQ at age 6 years was estimated for blood Pb level peaking at age 5 years.
Source: Hornung et al. (2009)

Figure 4-14 Estimated FSIQ for three different temporal patterns in blood Pb level from ages 2 to 6 years in Rochester and Cincinnati cohorts.

Results from both the Boston and Yugoslavia cohorts demonstrated cognitive function decrements in children with increases in blood Pb level from the prenatal to postnatal period (Wasserman et al., 2000a; Bellinger et al., 1990) (Table 4-15). In the Boston cohort, children with high prenatal cord (≥ 10 µg/dL) and high concurrent (≥ 10 µg/dL) blood Pb level had a decrease in cognitive function from age 24 to 57 months (Bellinger et al., 1990). In contrast, children with high prenatal blood Pb but low concurrent (<3 µg/dL) blood Pb level had an increase in cognitive function from age 24 to 57 months (Table 4-15). Findings from this study indicated that by age 5 years, children with higher prenatal blood Pb levels appeared to recover the Pb-associated decrements in cognitive function unless concurrent blood Pb levels remained high. The investigators also demonstrated that optimal sociodemographic characteristics (e.g., higher HOME score, SES, maternal IQ and age, female) also protected against decrements in cognitive function associated with higher postnatal blood Pb levels. Similarly, environmental enrichment with items such as toys, tunnels, and running wheels in cages given after gestational-lactational Pb exposure was found to protect rats from deficits in spatial learning in adulthood (Guilarte et al., 2003). The Yugoslavia study examined in detail during what periods of postnatal development were increases in blood Pb level associated with FSIQ decrements. Children with at least a 50% increase in blood Pb level from the prenatal period only up to age 2 year (early postnatal increase) and children with increases to age 2 years and to 7 years (early and later postnatal increase) had lower FSIQ compared with children with no increase in blood Pb level from the prenatal or postnatal period (Wasserman et al., 2000a) (Table 4-15). The decrease in FSIQ was greater among children with both early and later postnatal increases in blood Pb level. Collectively, these results from the Boston and Yugoslavia cohorts suggest that cognitive development is not fixed early in childhood and can be affected negatively or positively by postnatal Pb exposures or other factors that influence cognitive function.

Chen et al. (2005a) found a stronger influence of higher concurrent than age 2 year blood Pb levels on FSIQ at age 7 years among children participating in a multi-city chelation trial. Children with higher concurrent blood Pb levels (≥ median 7.2 µg/dL) had lower FSIQ at age 7 years, regardless of whether blood Pb level at age 2 years was low or high (less than or greater than the median of 24.9 µg/dL, respectively). Blood Pb levels at ages 2 and 7 years were weakly correlated (r = 0.27). In another analysis of the same study population (Liu et al., 2002) plus analysis of another chelation trial, results directly demonstrated increases in cognitive function in children with decreases in blood Pb levels over follow-up of 6 to 36 months (Ruff et al., 1993). Liu et al. (2002) found such an association in the group not receiving chelation. Because these children had initial high blood Pb levels (20-55 µg/dL) at ages 1 to 7 years, the findings may have limited generalizability to the general population of children currently living in the U.S.

Examining an older cohort (Port Pirie, Australia) at ages 11-13 years, Tong et al. (1998) found some evidence of persistence of effects of higher early-life blood Pb level (Table 4-15). This conclusion was based on the analysis of groups of children with different degrees of decline in blood Pb levels between ages 2 and 11-13 years. Although the mean blood Pb level in the study population declined overall from 21.2 µg/dL at age 2 years to 7.9 µg/dL at age 11-13 years, the magnitude of decline varied among children. The change in cognitive function between ages 2 and 11-13 years did not significantly differ between children with the largest decline (>16 µg/dL) in blood Pb level over that time period and children with the smaller decline (<10 µg/dL) (Table 4-15). Children with the largest decline in blood Pb level between ages 7 and 11-13 years had a slightly smaller decline in FSIQ over that time period. Thus, the findings indicated that cognitive function assessed in adolescents may be influenced by higher early-life blood Pb levels but also suggest some impact of more recent declines in blood Pb level. The results do not preclude an independent association with concurrent blood Pb level.

To conclude, the collective body of epidemiologic evidence does not strongly identify an individual critical lifestage or timing of Pb exposure with regard to neurodevelopmental effects in children. Cognitive function decrements and increases in externalizing and internalizing behaviors assessed at ages 3-24 years have been associated with prenatal, early childhood (e.g., age 1 or 2 year), lifetime average (to age 4-5, 7, or 11-13 years), and concurrent (at ages 3-17 years) blood Pb levels as well as with childhood tooth Pb levels. The identification of critical lifestages and time periods of Pb exposure is complicated further by the fact that blood Pb levels in older children, although affected by recent exposure, are also influenced by Pb stored in bone due to rapid growth-related bone turnover in children relative to adults. Thus, associations of neurodevelopmental effects with concurrent blood Pb level in children may reflect the effects of past and/or recent Pb exposures (Section 3.3.5.1). Evidence indicates that prenatal blood Pb levels are associated with mental development in very young children <age 2 years. However, in several studies, increases in postnatal (earlier childhood, lifetime average, concurrent) blood Pb levels were associated with larger cognitive function decrements in children ages 4-10 years than were increases in prenatal blood Pb levels (Table 4-14). These results suggest that per unit increase, postnatal Pb exposures that are reflected in concurrent or cumulative blood Pb levels or tooth Pb levels may have a larger magnitude of effect on cognitive function decrements as children age. Results from some studies indicated higher cognitive function in children at ages 1-8 years who had declines in blood Pb level over durations of 6 months to 5 years compared with children with smaller declines, no change, or increases in blood Pb levels (Hornung et al., 2009; Chen et al., 2005a; Liu et al., 2002; Ruff et al., 1993; Bellinger et al., 1990), providing some evidence that the effects of early Pb exposure on cognitive function are not fixed. These observations were made with declines from higher blood Pb levels at baseline (20-55

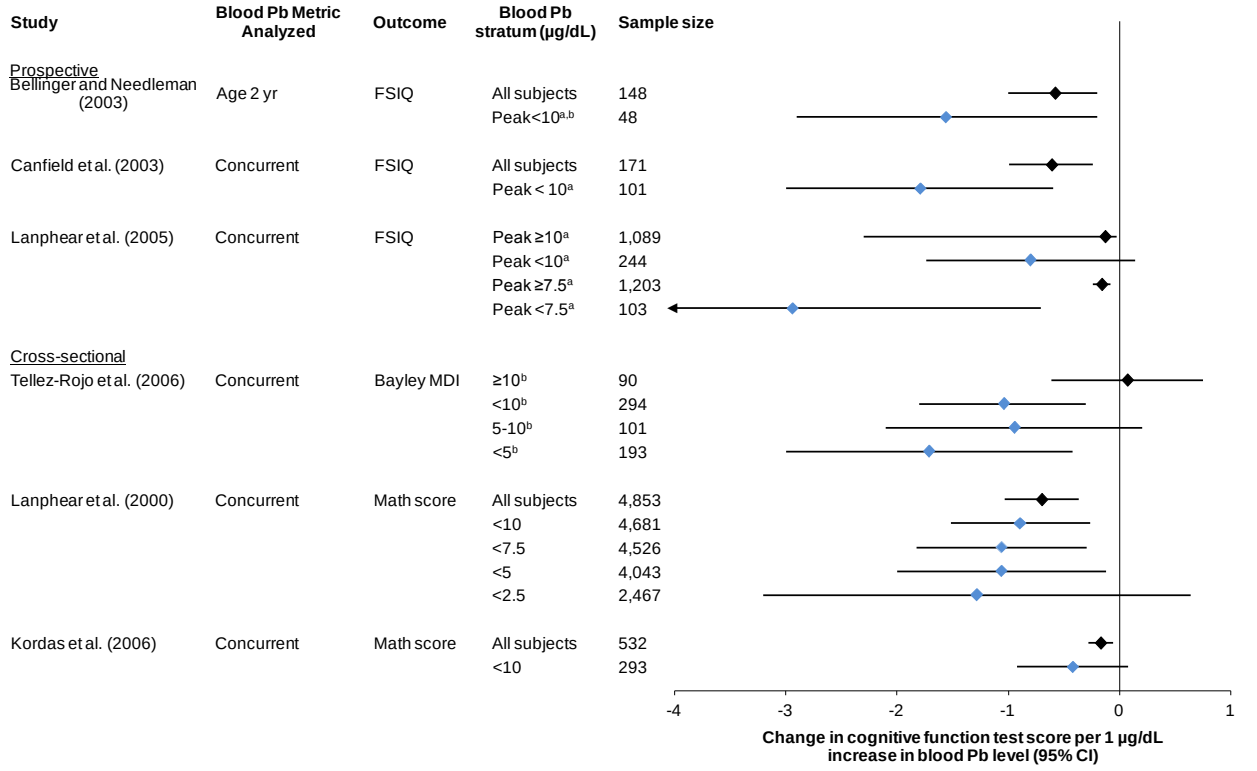
µg/dL) or larger declines over time (i.e., 8, 14 µg/dL) than those expected for most of the current population of U.S. children. Nonetheless, these findings are consistent with the understanding that the nervous system continues to develop throughout childhood. The epidemiologic evidence for associations of neurodevelopmental effects with multiple lifestages or time periods of Pb exposure, including more recent exposures, is supported by evidence in monkeys for Pb exposures during multiple lifestages and time periods, including infancy only, lifetime starting from birth, or lifetime starting during the juvenile period, inducing impairments in cognitive function when assessed between ages 6 and 10 years (Rice, 1992b; Rice and Gilbert, 1990a; Rice, 1990; Rice and Gilbert, 1990b; Rice and Karpinski, 1988).

4.3.12 Examination of the Pb Concentration-Response Relationship

With successive Pb AQCD and supplements, epidemiologic and toxicological studies find that progressively lower blood Pb levels are associated with effects on cognitive function and behavior. For example, among children, such effects were observed in association with blood Pb levels in the range of 10-15 µg/dL (for prenatal maternal or cord) in the 1986 Addendum (U.S. EPA, 1986c) and 1990 Supplement to the 1986 Addendum (U.S. EPA, 1990a) and 10 µg/dL and lower in the 2006 Pb AQCD (U.S. EPA, 2006b). Further, in the 2006 Pb AQCD, several individual studies and pooled analyses estimated a supralinear concentration-response relationship in children, i.e., greater decrements in cognitive function per unit increase in blood Pb level among children in lower strata of blood Pb levels compared with children in higher strata of blood Pb level (Figure 4-15 and Table 4-16). Some evidence in animals also indicates nonlinear concentration-response relationships for various endpoints, including those related to learning impairments. Some toxicological evidence points to larger absolute effects in lower Pb exposure groups (relative to control groups) than in higher exposure groups. However, these toxicological findings of U- or inverted U-shaped relationships are distinct from epidemiologic findings of supralinear relationships in that some toxicological relationships do not indicate Pb-induced impairments at higher exposure concentrations.

Several prospective epidemiologic studies found supralinear concentration-response relationships for concurrent, early childhood (age 2 year), and lifetime average (to age 5 years) blood Pb levels and cognitive function decrements in children ages 2-16 years. Most studies used a blood Pb level of 10 µg/dL to define lower and higher strata and reported mean or median blood Pb levels in the lower strata of 3-7 µg/dL (Bellinger, 2008; Canfield, 2008; Hornung, 2008; Téllez-Rojo, 2008). Except for the pooled analysis, the lower strata of blood Pb levels comprised >30% of the study population,

indicating that the blood Pb-cognitive function relationships calculated for the lower strata likely are not outliers or unrepresentative of the overall study population. Studies in the Boston and Rochester cohorts each examined different ages of blood Pb and FSIQ but lower blood Pb levels compared to other studies (means ~6 µg/dL), and a greater FSIQ decrement per unit increase in concurrent blood Pb level was found for children whose peak blood Pb levels < 10 µg/dL compared with all children examined (Bellinger and Needleman, 2003; Canfield et al., 2003a; Bellinger et al., 1992). A nonlinear blood Pb-FSIQ relationship also was indicated in pooled analyses of seven prospective studies involving a wider range of blood Pb levels (5th-95th percentiles 2.5-33.2 µg/dL) by statistical significance of blood Pb level modeled as quadratic and cubic functions (Lanphear et al., 2005) or better fit for a log-linear than linear model (Rothenberg and Rothenberg, 2005).



[a]Strata were defined by the peak blood Pb level measured in child at any point during follow up. [b]95% CI estimated from reported p-value.

Note: Results are presented first for prospective analyses then for cross-sectional analyses. FSIQ = full-scale IQ, MDI = mental development index. Effect estimates (concentration-response) are presented for a 1 µg/dL increase in blood Pb level. Black symbols represent effect estimates among all subjects or subjects in the higher blood Pb stratum. Blue symbols represent effect estimates in lower blood Pb strata.

Figure 4-15 Comparison of associations between blood Pb level and cognitive function among various blood Pb strata.

Table 4-16 Additional characteristics and quantitative results for studies presented in Figure 4-15.

Study	Study Population and Methodological Details[a]	Blood Pb Metric Analyzed (µg/dL)	Outcome	Blood Pb stratum (µg/dL)	Effect Estimate (95% CI)[b]
Bellinger et al. (1992) Bellinger and Needleman (2003) Bellinger (2008) U.S. EPA (2006b)	148 children followed from birth (1979-1981) to age 10 yr, Boston area, MA. **Prospective**. Recruitment at birth hospital. Participation by 59% of original cohort but 88% from age 5 yr. Participants had higher SES, HOME score. Linear regression model adjusted for HOME score (age 10 and 5), child stress events, race, maternal IQ, age, marital status, SES, sex, birth order, # residence changes before age 5 yr	Early childhood (age 2 yr) Mean (SD) **All subjects:** 6.5 (4.9) **Peak <10:** 3.8 (range: 1-9.3) Detection limit not reported	FSIQ WISC-R Age 10 yr	All 148 subjects 48 subjects peak <10	-0.58 (-1.0, -0.17) -1.6 (-2.9, -0.2)
Canfield et al. (2003a) Canfield (2008)	171 children born 1994-1995 followed from age 6 mo to 5 yr, Rochester, NY. **Prospective**. Recruitment from study of dust control. 73% nonwhite. Moderate follow-up participation but no selective attrition. Mixed effects models adjusted for child sex, Fe status, and birth weight, maternal race, education, IQ, income and prenatal smoking, HOME score	Concurrent Mean (SD) **All subjects:** 5.8 (4.1) **Peak <10:** 3.3 (range: 0.5-8.4) Detection limit = 1	FSIQ Stanford-Binet Age 5 yr	All 171 subjects 101 subjects peak <10	-0.61 (-0.99, -0.24) -1.8 (-3.0, -0.60)
Jusko et al. (2008)	174 children born 1994-1995 followed from age 6 mo to 6 yr, Rochester, NY. **Prospective.** Same cohort as above. High follow-up participation. Higher follow-up of nonwhite, higher peak blood Pb, lower maternal IQ. Generalized additive model with LOESS adjusted for sex, birth weight, transferrin saturation, maternal race, IQ, education, and prenatal smoking status, HOME score (6 yr), family income.	Peak (6 mo-6 yr) Mean (SD): 11.4 (7.3)	FSIQ WPPSI-R Age 6 yr	Peak 20-30, n not given Peak 10-20, n not given 96 subjects, peak 2.1-10	-0.15[c] -0.32[c] -1.2[c]

Table 4-16 (Continued): Additional characteristics and quantitative results for studies presented in Figure 4-15.

Study	Study Population and Methodological Details[a]	Blood Pb Metric Analyzed (µg/dL)	Outcome	Blood Pb stratum (µg/dL)	Effect Estimate (95% CI)[b]
Lanphear et al. (2005) Hornung (2008)	1,333 children pooled from Boston, Cincinnati, Cleveland, Mexico City, Port Pirie, Rochester, and Yugoslavia cohorts. **Prospective**. Large, uniform analysis pooling diverse cohorts. Included 84% of eligible children. Linear regression model adjusted for HOME score, birth weight, maternal IQ and education. Considered child sex, marital status, birth order, prenatal alcohol consumption and smoking status, maternal age.	Concurrent Mean (range) **Peak ≥ 10:** 13.9 (0.1-71.7) **Peak <10:** 4.3 (0.1-9.8) **Peak ≥ 7.5**: 12.9 (0.1-71.7) **Peak <7.5:** 3.2 (0.9-7.4)	FSIQ Various tests Ages 4.8-10 yr	1,089 subjects peak ≥ 10 244 subjects peak <10 1,203 subjects peak ≥ 7.5 103 subjects peak <7.5	-0.13 (-2.3, -0.03) -0.80 (-1.7, 0.14) -0.16 (-0.24, -0.08) -2.9 (-5.2, -0.71)
Tellez-Rojo et al. (2006) Tellez-Rojo (2008)	384 children followed from birth (1994-1995, 1997-1999) to age 2 yr, Mexico City, Mexico. **Cross-sectional.** Recruitment from prenatal clinic or birth hospital. Participants had higher maternal education, lower cord blood Pb. Linear regression model adjusted for sex, birth weight, maternal IQ. Considered maternal age and other unspecified factors.	Concurrent Mean (SD) **≥ 10:** NR **<10:** 4.3 (2.3) **5-10:** 6.9 (1.4) **<5**: 2.9 (1.1)	Bayley MDI Age 2 yr	90 subjects ≥ 10 294 subjects <10 101 subjects 5-10 193 subjects <5	0.07 (-0.61, 0.75)[c] -1.0 (-1.8, -0.25)[c] -0.94 (-2.1, 0.23)[c] -1.7 (-3.0, -0.42)[c]
Lanphear et al. (2000)	4,853 children ages 6-16 yr, NHANES III 1988-1994. **Cross-sectional**. Large study of multiple exposures and outcomes. Linear regression model adjusted for U.S. region, sex, race/ethnicity, reference adult education, poverty index ratio, marital status, serum ferritin level, serum cotinine level. Did not consider parental IQ or caregiving quality.	Concurrent Geometric mean (SD) All subjects: 1.9 (7.0) Subgroups: NR	Math Score WRAT Ages 6-16 yr	All 4,853 subjects 4,681 subjects <10 4,526 subjects <7.5 4,043 subjects <5 2,467 subjects <2.5	-0.70 (-1.0, -0.37) -0.89 (-1.5, -0.26) -1.1 (-1.8, -0.30) -1.1 (-2.0, -0.12) -1.3 (-3.2, 0.64)

Table 4-16 (Continued): Additional characteristics and quantitative results for studies presented in Figure 4-15.

Study	Study Population and Methodological Details[a]	Blood Pb Metric Analyzed (µg/dL)	Outcome	Blood Pb stratum (µg/dL)	Effect Estimate (95% CI)[b]
Kordas et al. (2006)	532 children ages 6-8 yr, Torreon, Mexico **Cross-sectional**. Recruitment at schools. Residence near metal foundry. High participation. Linear regression model adjusted for sex, age, hemoglobin, family possessions, forgetting homework, house ownership, crowding, maternal education, birth order, family structure, urine arsenic, tester, school. Did not consider parental IQ or caregiving quality.	Concurrent Mean (SD) All subjects: 11.4 (6.1) < 10: NR	Math score Achievement test Ages 6-8 yr	All 532 subjects 293 subjects <10	-0.17 (-0.28, -0.06) -0.42 (-0.92, 0.08)

FSIQ = Full-scale IQ, WISC-R = Wechsler Intelligence Scale for Children-Revised, WPPSI-R = Wechsler Preschool and Primary Scale of Intelligence-Revised, MDI = Mental Developmental Index, NR = Not reported, WRAT = Wide Range Achievement Test.

[a]Studies are grouped by strength of study design, representativeness of the study population characteristics and blood Pb levels examined, and extent of consideration for potential confounding. There is not necessarily a continuum of decreasing strength across studies.

[b]Most effect estimates are derived from linear models and are presented for a 1 µg/dL increase in blood Pb level.

[c]Results not included in Figure 4-15 because nonparametric analysis did not produce 95% CIs for various strata of blood Pb levels.

A few cross-sectional studies demonstrated larger Pb-associated decreases in cognitive function in groups with concurrent blood Pb levels <5 µg/dL than groups with higher levels. Tellez-Rojo et al. (2006) estimated a larger decrement in age 2 year Bayley MDI per unit increase in blood Pb level for children with concurrent blood Pb levels <5 µg/dL compared with children with blood Pb levels 5-10 µg/dL, and >10 µg/dL (Figure 4-15 and Table 4-16). However, it is not clear what the implications of age 2 year MDI results may be on cognitive function at later ages. Among children ages 6-16 years participating in NHANES 1988-1994, Lanphear et al. (2000) found larger decrements in reading and math skills and memory per unit increase in blood Pb level in children with concurrent blood Pb levels <2.5 µg/dL than children with levels <5 µg/dL, <7.5 µg/dL, <10 µg/dL, and all subjects. However, these children born 1972-1989 likely had higher blood Pb levels (Figure 3-18) and Pb exposures earlier in childhood, which may have contributed to the observed associations.

Several (Min et al., 2009; Jusko et al., 2008; Schnaas et al., 2006) but not all (Palaniappan et al., 2011) recent studies found evidence for a nonlinear blood Pb-cognitive function relationship in nonparametric regression analyses using splines or LOWESS with smoothing parameters that did not produce quantitative results for individual blood Pb groups. Similar to the pooled analyses of the seven prospective cohorts, these relationship were evaluated for a wide range of blood Pb levels. In the Rochester and Mexico City cohorts, the blood Pb-FSIQ relationship was more negative for children with lower blood Pb levels, specifically for peak blood Pb levels 2.1-10 µg/dL (overall range: 2.1-46 µg/dL) in the Rochester cohort at age 6 years (Jusko et al., 2008) and for prenatal maternal week 28-36 blood Pb levels <6 µg/dL (overall 5th-95th percentile 2.5-25 µg/dL) in the Mexico City cohort at ages 6-10 years (Schnaas et al., 2006). In a formal test of nonlinearity, Schnaas et al. (2006) found the nonlinear blood Pb term to fit the data better than a linear term. Among 278 children of age 4 years (blood Pb range: 1.3-24 µg/dL) who had high prenatal alcohol and drug exposure, Min et al. (2009) reported a p-value of 0.19 for a restricted cubic spline term for blood Pb level and described the covariate-adjusted concurrent blood Pb level-FSIQ curve to be more negative at blood Pb levels 1.3-7 µg/dL. Among 814 children in India ages 3-7 years, Palaniappan et al. (2011) mostly found linear associations between concurrent blood Pb level (range: 2.6-41 µg/dL) and indices of cognitive function. The exception was visual-motor skills, for which a greater blood Pb-associated decline was found with blood Pb levels >30 µg/dL. The linearity versus nonlinearity of the blood Pb-FSIQ concentration-response relationship within a lower, more narrow range of blood Pb levels has not been examined in detail.

Few studies of adults have examined whether the relationship between blood or bone Pb level and cognitive function is described better with a linear or nonlinear function. In

analyses of adults in NHANES, only log-linear models were used to fit the data (Krieg et al., 2010; Krieg and Butler, 2009; Krieg et al., 2009). Nonlinearity in the BMS and NAS cohorts was examined with the use of quadratic terms, penalized splines, or visual inspection of bivariate plots (Bandeen-Roche et al., 2009; Wang et al., 2007a; Weisskopf et al., 2007b; Shih et al., 2006). There was some evidence for nonlinearity in prospective analyses of the NAS cohort (Figure 4-7 and Figure 4-8), but not all results indicated greater declines in cognitive function per unit increase in bone Pb level in the lower bone Pb groups. Wang et al. (2007a) found that among NAS men with an HFE gene variant, there was a larger decline in MMSE score per unit increase in tibia Pb level at higher tibia Pb levels (Figure 4-8). In the BMS cohort, linear relationships were indicated for various tests of cognitive function by a statistically nonsignificant quadratic term (Shih et al., 2006) or spline (Bandeen-Roche et al., 2009) for tibia Pb level.

Although not specific to Pb exposure, attenuation of concentration-response relationships at higher exposure or dose levels has been reported in the occupational literature, and explanations have included greater exposure measurement error and saturation of biological mechanisms at higher levels and larger proportions of at-risk populations at lower exposure levels (Stayner et al., 2003). Hypotheses for nonlinearity in the relationship between Pb and cognitive function include a lower incremental effect of Pb due to covarying risk factors such as low SES, poor caregiving environment, higher exposure to other environmental risk factors (Schwartz, 1994), different mechanisms operating at different exposure levels, and confounding by omitted or misspecified variables. The contribution of these factors to the supralinear relationship observed between blood Pb levels and cognitive function in children has not been examined in many epidemiologic studies to date. Some studies found that risk factors such as SES, parental education, and parental caregiving quality explain a greater proportion of variance in cognitive function than does blood Pb level (Wasserman et al., 1997; Greene et al., 1992). Recently, among 57,678 fourth grade children across North Carolina, Miranda et al. (2009) found that lower parental education and enrollment in a free/reduced fee lunch program accounted for larger decrements in EOG scores than did blood Pb level across the various quantiles of EOG score distribution (Figure 4-6). In a meta-analysis, the blood Pb-FSIQ relationship was more negative in studies of lower SES populations than in studies of higher SES populations (Schwartz, 1994).

Few studies have examined effect modification of the blood Pb level-cognitive function relationship by covarying risk factors such as sociodemographic factors, and the limited evidence is inconclusive. None of these studies examined effect modification within specific strata of blood Pb levels. Among children in the Boston cohort in the higher cord blood Pb level group (>10 µg/dL), cognitive function between ages 24 and 57 months increased in the group that was female and had higher HOME score, SES, and maternal

IQ (Bellinger et al., 1990). In the Port Pirie, Australia cohort, larger blood Pb-associated FSIQ decrements were found in groups with lower SES at age 11-13 years (Tong et al., 2000) but not lower HOME score at age 4 years (McMichael et al., 1992). Similar effects of blood Pb level on FSIQ were estimated in the higher SES Boston cohort and the lower SES Rochester cohort (Table 4-3). Overall, evidence does not clearly indicate whether the blood Pb-cognitive function relationship is modified by factors such as SES or other sociodemographic characteristics or whether these differences can explain the observed nonlinear concentration-response relationship.

Results from the pooled analysis by Rothenberg and Rothenberg (2005) do not indicate that residual confounding by covariates explains the nonlinear blood Pb-FSIQ relationship. Modeling maternal IQ, HOME score, maternal education, and birthweight as spline functions (df = 2) did not significantly improve model fit either with a linear blood Pb term or log blood Pb term, which indicated that the improved model fit with log-specification of blood Pb level was not influenced by the modeling of covariates as linear or nonlinear functions.

Bowers and Beck (2006) postulated that a supralinear concentration-response function necessarily will be found in a model with a log-normally distributed independent variable and a normally distributed outcome variable. However, as discussed in the 2006 Pb AQCD, this modeling strategy was not employed in the epidemiologic analyses showing a supralinear concentration-response function. FSIQ scores generally were not forced into a normal distribution. Normalized FSIQ scores were not the basis for individual findings from four of the seven studies included in the pooled analysis by Lanphear et al. (2005) or the results pooling the seven cohorts (Hornung et al., 2006). Further, a log-linear model (a linear relationship between IQ and the log of blood Pb) provided a better fit of the pooled data.

Results from prospective analyses in the Boston and Rochester cohorts for associations between blood Pb level as a continuous variable and FSIQ in groups of children in the lower segment of the population blood Pb distribution have not identified a threshold in the range of blood Pb levels examined. In the Boston cohort, higher age 2 year blood Pb levels were associated with lower FSIQ at age 10 years in children with blood Pb levels 1-9.3 µg/dL whose peak blood Pb levels were less than 10 µg/dL (Bellinger, 2008; Bellinger and Needleman, 2003). Schwartz (1994) explicitly assessed evidence for a threshold in the Boston cohort data by regressing FSIQ and blood Pb level on covariates such as age, race, maternal IQ, SES, and HOME score and fitting a nonparametric smoothed curve to the residuals of each regression model (variation in FSIQ or blood Pb level not explained by covariates). A 7-point decrease in FSIQ was found over the range of blood Pb residuals below 0 (corresponding to the mean blood Pb level of 6.5 µg/dL),

indicating an association between blood Pb level and FSIQ well below the mean blood Pb level. Results from the Rochester cohort did not identify a threshold either. Higher concurrent blood Pb levels (range 0.5-8.4 µg/dL, detection limit = 1 µg/dL) were associated with lower FSIQ at ages 3 and 5 years in children with peak blood Pb levels <10 µg/dL (Canfield et al., 2003a). A threshold also was not identified for the association between concurrent blood Pb level and MDI score at age 2 years among children in Mexico City (Téllez-Rojo et al., 2006). Higher blood Pb level was associated with a lower MDI score in the group of children with blood Pb levels <5 µg/dL (range 0.8-4.9 µg/dL) (Téllez-Rojo, 2008). Other results based on nonparametric regression analyses did not identify a threshold for Pb-associated cognitive function decrements in children, but results have weaker implications because the blood Pb levels in the examined populations were higher (e.g., minimum 2.1 µg/dL, 5th percentiles 2.5 µg/dL) (Jusko et al., 2008; Schnaas et al., 2006; Lanphear et al., 2005) than those in most current U.S. children.

In conjunction with downward trends in population blood Pb distributions (Figure 3-16), more sensitive quantification methods have improved measurement of blood Pb levels in the lower portion of the population distribution (e.g., in NHANES, from 0.6 µg/dL in 1999-2002 to 0.025 µg/dL in 2003-2004). Consequently, the examination of groups of children (ages 4-15 years) with lower blood Pb levels, in the range of <1 to 16 µg/dL, has indicated Pb-associated cognitive function decrements or increases in externalizing behaviors at lower blood Pb levels (Cho et al., 2010; Kim et al., 2009b; Miranda et al., 2009; Braun et al., 2008; Miranda et al., 2007a; Braun et al., 2006). In the studies examining concurrent blood Pb levels, the potential contribution of higher past Pb exposures obscures assessment of a threshold. For example, Braun et al. (2008; 2006) found higher odds of parental reports of conduct disorder and ADHD among children ages 4-15 years with concurrent blood Pb levels ~1.0 µg/dL compared with children with blood Pb levels <0.8 µg/dL (6 or 7% below detection limit of 0.3 µg/dL). However, children likely had higher early childhood blood Pb levels, and adolescents in the study were born in the 1970s during the use of leaded gasoline.

Results from Miranda et al. (2009) did not identify a threshold with the examination of blood Pb levels measured between ages 6 and 36 months during 1995-1999. Among 57,678 children in North Carolina, lower 4th grade EOG scores were found in children in the blood Pb level category of 2 µg/dL (exact values not reported) compared with children in the blood Pb level category of 1 µg/dL, which included children with blood Pb levels at or below the detection limit. Other analyses of blood Pb level as a categorical variable did not clearly address the identification of a threshold for the blood Pb-cognitive function relationship. However, these analyses of categories that encompass a wider range of blood Pb levels may be less sensitive. Cognitive function decrements

were limited to children ages 7-8 years in the U.K. with age 30-month blood Pb levels 5-10 µg/dL (compared with blood Pb levels 0-2 µg/dL) (Chandramouli et al., 2009) and children ages 6-10 years in New England with concurrent blood Pb levels 5-10 µg/dL (compared with blood Pb levels 1-2 µg/dL) (Surkan et al., 2007).

Some toxicological studies found nonlinear relationships between Pb exposure and effects related to impaired learning and memory in animals, although these differ from the nonlinear relationships found in epidemiologic studies of children in that nonlinearity in animal studies has been demonstrated as lower and higher Pb exposures having effects in opposite directions (U- or inverse U-shaped curves). Epidemiologic results for nonlinearity indicate negative effects over the entire blood Pb distribution examined. Results summarized across multiple studies in multiple species demonstrated that lower Pb exposures increased FI response rates relative to controls, and higher Pb exposures decreased FI response rates (Cory-Slechta, 1994). Increased FI response rates indicate impaired learning by reflecting the impaired ability of animals to respond according to a fixed schedule of reinforcement (Section 4.3.2.3). Consistent with previous findings, Rossi-George et al. (2011) found that 50 ppm lifetime Pb exposure increased FI overall rate in rats tested at age 2 months whereas 150 ppm Pb exposure did not affect FI responses. Nonlinear effects of Pb on learning were not observed with longer duration exposures (e.g., 8-11 months) (Cory-Slechta, 1990). The nonlinear effects of Pb on impaired learning were supported by evidence in animals indicating that lower and higher Pb exposures differentially activate underlying mechanisms. Gilbert et al. (1999) found reduced LTP magnitude in adult rats exposed to 1,000-5,000 ppm but not 10,000 ppm Pb acetate in drinking water from GD16. LTP is one indication of synaptic plasticity (Section 4.3.10.4), which is considered to be a major cellular mechanism underlying learning and memory. Synaptic plasticity also is affected by glutamatergic neurotransmission via its NMDA receptor (Section 4.3.10.8), and reduced glutamate release in the hippocampus was found in adult rats exposed to Pb acetate from GD15-GD16 with blood Pb levels 27-40 µg/dL but not with blood Pb levels of 62-117 µg/dL (Lasley and Gilbert, 2002).

Dopaminergic neurotransmission is involved in many CNS processes including cognition, behavior, and motor function. The shape of the Pb-DA concentration-response relationship varied among rodent studies. Some studies found that lower Pb exposures (e.g., 27, 50 ppm) did not affect or increased DA activity relative to controls and higher Pb exposure (109, 150, 250 ppm) (Leasure et al., 2008; Virgolini et al., 2005; Lewis and Pitts, 2004). However, sometimes in the same study, in other regions of the brain or under some conditions, higher Pb exposures (109, 150 ppm) increased or impaired DA activity (Leasure et al., 2008; Virgolini et al., 2005). These differential responses of DA may be related to the diverse CNS effects of DA in different regions of the brain. For

example, the increased forebrain dopamine turnover with 27 ppm gestational/lactational Pb acetate exposure was accompanied by less spontaneous activity in male mice compared with male mice exposed to 109 ppm Pb (Leasure et al., 2008).

In vitro results indicated differential effects on calcineurin enzyme activity, with inhibited activity resulting from higher Pb exposure (>2×10^{-4} µM) and stimulated activity from lower Pb exposure (Kern and Audesirk, 2000). While calcineurin activity has been found to modulate learning, LTP, and behavior in animals, lower calcineurin activity has been associated with both impaired and improved performance on learning and memory tasks (Zeng et al., 2001). Thus, it is uncertain whether altered calcineurin activity can explain the nonlinear relationships observed between Pb exposure and impaired learning in animals. At lower concentrations, Pb may displace calcium at its binding sites on calmodulin and by acting as a calmodulin agonist at the catalytic A subunit of calcineurin stimulate calcineurin activity. At higher Pb exposure, Pb may bind directly to a separate calcium-binding B subunit, override the calmodulin-dependent effect and turn off the activity of calcineurin. Lasley and Gilbert (2002) found that 2,000 ppm but not 5,000 or 10,000 ppm Pb acetate exposure of rats (in drinking water starting at GD15-GD16) inhibited glutamate release by acting as a calcium mimetic.

Some toxicological studies have found nonlinear relationships for non-cognitive outcomes in animals. U-shaped Pb concentration-response relationships were found for spontaneous motor activity level and latency to fall from rotarod (Leasure et al., 2008). Inverted U-shaped relationships were found for hippocampal neurogenesis related to Pb exposure duration (24 hours not 28 days after BrdU injection) or concentration (Fox et al., 2008; Gilbert et al., 2005). Evidence also points to differences in hormone production by Pb exposure concentration. In male mice with long-term Pb exposure (PND21-9 months of age), basal corticosterone levels were significantly lower with 50 ppm Pb than with 150 ppm Pb or controls (Virgolini et al., 2005). The visual system in animals also has shown to be affected differentially by lower versus higher Pb exposure (GD1-PND10, pup blood Pb levels 10-12, 24-27, and 42-46 µg/dL). Inverted U-shaped concentration-response curves were observed for rod photoreceptor numbers in the retina (Giddabasappa et al., 2011) and retinal layer thickness (Fox et al., 2010). These dichotomous histological findings may give insight to the complex Pb-associated changes in ERG wave amplitudes that vary by exposure timing and dose (Section 4.3.6.2).

To conclude, several studies found a supralinear blood Pb-cognitive function concentration-response relationship in children but not adults based on comparisons of effect estimates in lower and higher strata of blood Pb level and results from nonparametric regression modeling. Explanations for this supralinear relationship have not been well characterized by epidemiologic studies. Evidence from the prospective

studies in the Boston and Rochester cohorts has not identified a threshold for Pb-associated cognitive function decrements in the range of blood Pb levels examined. Increases in childhood blood Pb levels ranging from those at or below the detection limit of 1.0 μg/dL to 9.3 μg/dL (means: 3.3 and 3.8 μg/dL) were associated with cognitive function decrements at ages 3 to 10 years in children whose peak blood Pb levels were less than 10 μg/dL (Bellinger, 2008; Canfield, 2008; Bellinger and Needleman, 2003; Canfield et al., 2003a). Further, a recent study found an association between higher blood Pb levels measured at ages 6-36 months (1995-1999) and lower 4th grade EOG scores in 57,678 children in North Carolina with blood Pb levels at or below the detection limit of 1 μg/dL to 16 μg/dL (Miranda et al., 2009). Concurrent blood Pb levels in the range of 0.8-9.8 μg/dL (detection limit not reported) were associated with MDI decrements in children age 2 years in Mexico City (Téllez-Rojo, 2008; Téllez-Rojo et al., 2006). The lack of a reference population with blood Pb levels reflecting pre-industrial Pb exposures limits the ability to identify a threshold. Analysis of ancient bones in pre-industrialized societies suggests that "background" blood Pb levels in pre-industrial humans may have been more than 50-fold lower than those of the current U.S. population (Flegal and Smith, 1992) and lower than the levels at which neurodevelopmental effects have been examined. Thus, the current evidence does not preclude the possibility of a threshold for neurodevelopmental effects in children existing with lower blood levels than those currently examined. While distinct from supralinear relationships observed in epidemiologic studies, some toxicological studies showed that lower Pb exposures (e.g., 50 ppm in drinking water) induced learning and memory impairments in animals compared to control exposures or higher Pb exposures (e.g., 150 ppm). Additional toxicological evidence suggests that differentially activated mechanisms at lower and higher Pb exposures such as reduced LTP and hippocampal glutamate release with lower Pb exposures may provide explanation for the impaired learning and memory observed with lower Pb exposures in animals.

4.3.13 Confounding in Epidemiologic Studies of Nervous System Effects

In addition to Pb exposure, many factors influence cognitive function and behavior in children, including parental IQ and education, SES of the family, quality of the caregiving environment, and other environmental exposures (Wasserman and Factor-Litvak, 2001). These other risk factors often are correlated with blood, tooth, and bone Pb levels, thus, a major challenge to observational studies examining associations of Pb biomarker levels with cognitive function and behaviors in children has been the assessment and control for potential confounding factors. By definition, a confounder is associated with both the independent variable and the outcome and consequently has the

potential to bias the association between the independent variable of interest and the outcome. Most epidemiologic studies of Pb biomarkers in children have examined potential confounding by parental IQ and SES-related variables such as parental education, household income, and the Hollingshead Four-Factor Index of Social Position, which incorporates education and income of both parents. Fewer but still several studies have examined confounding by quality of the caregiving environment (i.e., HOME score), birth weight, and smoking exposure. A relatively smaller number of studies have considered nutritional status, other environmental exposures, parental substance abuse, or parental psychopathology. Studies have varied with respect to the number of potential confounding factors examined, with some studies considering multiple SES-related factors and other studies focusing on a smaller set. The extent of confounding by a particular factor likely varies across studies, depending on the population examined. Thus, the impact of adjustment for specific covariates on the Pb effect estimate also likely varies across studies.

Various methods have been used to control for potential confounding: examining a population relatively homogeneous in SES, examining populations in which factors are not correlated, conducting multivariate regression, characterizing the change in the Pb biomarker effect estimate with adjustment for a covariate, and examining associations in different strata of a covariate. The evidence derived from each of these control strategies is discussed below. No single method is without limitation, and adjustment for SES is difficult as it is highly correlated with Pb exposure and there is no single variable that fully represents SES. Residual confounding also is likely by factors not considered. The combination of evidence from prospective studies that considered several well-characterized potential confounding factors plus evidence that Pb exposure impairs cognitive function and behavior in animals, in particular, visual-spatial memory, executive function, and response inhibition, which are also found to be affected in children, increase confidence that the associations observed between Pb biomarker levels and neurodevelopmental effects in children represent a relationship with Pb exposure.

In the Boston prospective study, potential confounding by SES was reduced by study design and statistical adjustment for SES. The study subjects were from middle- to upper-middle-class families, a majority with married, college-educated parents. Hence, the potential for confounding by SES in this study was considerably less compared to other studies examining similar outcomes. In this cohort, higher prenatal cord and concurrent blood Pb levels were associated with FSIQ decrements at age 57 months, and higher age 2 year and age 57 month blood Pb levels were associated decrements in FSIQ and executive function at age 10 years (Stiles and Bellinger, 1993; Bellinger et al., 1992; Bellinger et al., 1990). In contrast, blood Pb levels measured at various ages were weakly associated with cognitive function decrements at age 7 years in the Sydney, Australia

cohort of middle-SES children (i.e., 20% mothers with > high school education) (Cooney et al., 1991). However, a relationship between Pb exposure and cognitive function decrements is supported by a similar magnitude of blood Pb-associated FSIQ decrement found in the Boston and the low-SES Rochester cohort (majority of mothers with < college education and annual income <$15,000), with adjustment for similar covariates (Figure 4-2 and Table 4-3) (Canfield et al., 2003a). For some outcomes, larger effects were estimated in the Boston cohort than in other cohorts.

Blood Pb levels also were associated with cognitive function decrements in populations in which higher blood Pb levels were not correlated with lower SES (Factor-Litvak et al., 1999; Bellinger et al., 1987). In the Yugoslavia cohort, blood Pb levels at age 4 years were higher in groups with higher maternal education, maternal IQ, and HOME score in the city near Pb sources and were lower in the distant city. Among all children, higher blood Pb level was associated with lower FSIQ and learning and memory scores and with higher ratings of internalizing behaviors (Factor-Litvak et al., 1999). In the Boston cohort, parental education, social class, HOME score, and birth outcomes were similar among low (<3 µg/dL), medium (6-7 µg/dL), and high (≥ 10 µg/dL) cord blood Pb level groups. Further, adjusting for these and other demographic factors, Bellinger et al. (1987) found that children in the high cord blood Pb group had a 4.8-point lower Bayley MDI score at age 2 years than did children in the low cord blood Pb group.

The primary method used by epidemiologic studies to control for potential confounding, in particular recent studies of children with blood Pb levels more similar to current U.S. levels, has been multivariate regression. Some studies modeled a set of covariates based on a priori evidence, whereas others selected specific covariates based on their association with the outcome in a model with all potential covariates and/or a greater than 10% change in the blood Pb level effect estimate. Studies also varied in the number of potential confounding factors included in models. Some included multiple SES-related variables, whereas others analyzed one or two factors. Regardless of the method used to select model covariates or the number of covariates included, studies consistently found associations of higher blood Pb level with cognitive function decrements and poorer behavior. The evidence suggests that confounding by particular factors may vary across populations and increases confidence that the associations observed with neurodevelopmental effects in children represent a relationship with Pb exposure.

The consistency in finding associations across populations with different SES and co-exposures and across studies examining different covariates was reinforced in pooled and meta-analyses (Marcus et al., 2010; Lanphear et al., 2005; Schwartz, 1994). Pooling data from seven international prospective cohorts, Lanphear et al. (2005) found similar FSIQ decrements per log increase in blood Pb level (-2.6% to +8.6% difference) by

excluding one study at a time. These results indicated a relatively robust pooled estimate despite between-study differences in population characteristics, including SES. In a meta-analysis, Schwartz (1994) found a relatively narrow range of blood Pb-FSIQ effect estimates among studies despite large between-study differences in the correlation between blood Pb level and SES. A wider range of effect estimates would be expected if omitted SES factors confounded the association. A recent meta-analysis of the association between blood Pb level and conduct problems from earlier and recent studies of children found that adjustment for SES and home environment did little to attenuate the association (Marcus et al., 2010).

Among the several studies that provided both unadjusted and adjusted effect estimates, most indicated that blood Pb level was a statistically significant predictor of cognitive function (e.g., FSIQ, executive function, learning, memory) in children ages 5-10 years before and after adjusting for potential confounders. Although most effect estimates changed by 20-50% in multivariate models, most remained within the 95% CI of the unadjusted estimate (Min et al., 2009; Kordas et al., 2006; Schnaas et al., 2006; Canfield et al., 2003a; Dietrich et al., 1993b; Bellinger et al., 1992). Such observations were made in previous analyses of the Boston and Rochester cohort with mean blood Pb levels 6.5 and 5.8 µg/dL, respectively, with adjustment for SES, maternal IQ and education, and HOME score (Canfield et al., 2003a; Bellinger et al., 1992). These analyses also adjusted for or considered potential confounding by indicators of nutritional status. Recent studies of children of a similar age range and mean blood Pb levels also found statistically significant associations (as indicated by correlation and/or regression coefficients) between blood Pb level and cognitive function before and after adjustment for similar covariates (Min et al., 2009; Chiodo et al., 2007); however, because of the high prevalence of prenatal alcohol or drug use in these populations, their results may be less generalizable than results from the Rochester and Boston cohorts.

Blood Pb level also was a statistically significant predictor of cognitive function after adjustment for covariates such as maternal education and IQ, SES, and HOME score in with children with higher mean blood Pb levels, 8-14 µg/dL (Kordas et al., 2006; Schnaas et al., 2006; Tong and Lu, 2001; Dietrich et al., 1993b). Exceptions include multiple analyses of the Cleveland cohort, in which blood Pb level was estimated to have a weak and imprecise or null effect after adjustment for potential confounding factors (Greene et al., 1992; Ernhart et al., 1989; Ernhart et al., 1988). Analyses of the Cleveland cohort considered similar potential confounding factors as other studies. HOME score was the major factor accounting for the attenuation of the Pb effect estimate in the Cleveland cohort. An analysis of the Yugoslavia cohort, which adjusted for most of the same covariates as the Cleveland analyses reported larger magnitude blood Pb-cognitive function associations in covariate-adjusted models (Factor-Litvak et al., 1999). The

collective findings in children indicate potential confounding by the SES-related and demographic factors examined in the literature base but also demonstrate that blood Pb level is an independent predictor of cognitive function decrements in children with adjustment for these factors.

A challenge to separating the effects of Pb exposure from those related to SES and parental caregiving quality is their frequently high correlation with blood Pb levels. In such cases, it is difficult to know how much variation in the outcome to attribute to each of the risk factors (Needleman and Bellinger, 2001). For example, due to the high correlation between blood Pb level and SES, a model that includes SES may underestimate the Pb effect because some of the variance in outcome due to Pb is mistakenly attributed to the variance due to SES. This misattribution may be exacerbated when multiple correlated variables are included in the same model (i.e., overcontrol). The relationships observed for Pb biomarker levels with SES and parental caregiving quality may indicate that they are proxies or determinants of Pb exposure rather than a confounder of the association of interest. Lower SES in urban children is closely linked to residence in older, poorer condition housing that, in turn, may increase exposure of children to environmental Pb and risk of cognitive function decrements (Clark et al., 1985). In such cases where SES is a determinant of Pb exposure, statistical adjustment for SES will result in overcontrol of the Pb effect (Bellinger, 2004a). This type of overcontrol could apply to results from the New Zealand cohort, which were adjusted for residence in older wooden housing, which is associated with higher exposure to Pb paint and accumulated dust and soil and higher child tooth Pb levels (Fergusson et al., 1988a, b). However, even in models with older wooden housing, Pb remained a statistically significant predictor of poorer reading skills and teacher ratings of school performance. SES has been shown to be an effect modifier of the Pb-child cognitive function relationship in some populations. Larger blood Pb-associated decreases in cognitive function were found with lower SES in some studies (Ris et al., 2004; Tong et al., 2000; Bellinger et al., 1990) and higher SES in a meta-analysis (Schwartz, 1994). In cases of effect modification, potential confounding by SES is less likely.

In summary, the collective epidemiologic evidence consistently demonstrates associations of higher blood and tooth Pb levels with cognitive function decrements and poorer behavior in children. These associations have been observed in diverse populations in the U.S., Mexico, Europe, Asia, and Australia. Associations have been observed across studies that use different methods to control for confounding and adjust for different potential confounding factors but commonly, maternal IQ and education, SES, and HOME score. Several studies have found associations with additional adjustment for smoking exposure, child birth outcomes, and nutritional factors. No single method to control for potential confounding is without limitation, and there is potential

for residual confounding by unmeasured factors. However, the consistency of findings among different populations and study methods with consideration of several well-characterized potential confounding factors as described above increases confidence that the associations observed between Pb biomarker levels and neurodevelopmental effects in children represent a relationship with Pb exposure. Biological plausibility is provided by the coherence with extensive evidence in animals with Pb exposures that produce blood Pb levels relevant to this ISA and that is not subject to confounding by factors such as social class and correlated environmental factors. Further, Pb exposure has been shown to induce decrements in visual-spatial memory, rule learning and reversal, and response inhibition, which also have been associated with blood or tooth Pb levels in children. Additional support for the epidemiologic evidence is provided by extensive toxicological evidence describing modes of action for Pb-induced cognition and behavioral problems, including changes in neurogenesis, synaptic pruning, and neurotransmitter function in the hippocampus, prefrontal cortex, and nucleus accumbens of the brain (Section 4.3.10).

4.3.14 Public Health Significance of Associations between Pb Biomarkers and Neurodevelopmental Effects

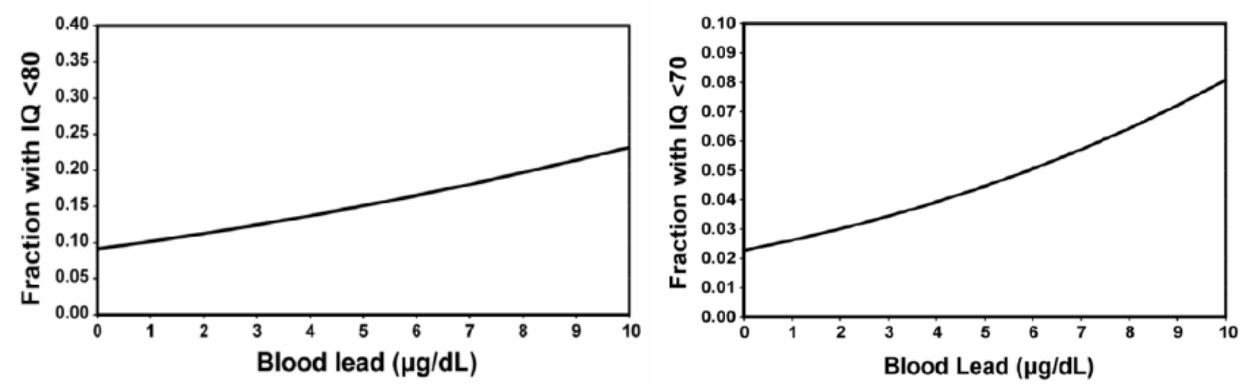
As described in Section 4.3.2.1, most studies found that a 1 µg/dL increase in blood Pb level was associated with decrements in FSIQ in school-aged children in the range of <1 to 2 points, depending on the model and blood Pb level range examined (Figure 4-2 and Table 4-3). Similarly, a 1 µg/dL increase in blood Pb level typically was associated with lower scores on tests of executive function (Table 4-8) and academic performance (Table 4-9), and higher ratings of behavioral problems (Figure 4-9, Table 4-11, Table 4-12) on the order of less than 1 standard deviation. Such findings prompt consideration of the public significance of blood Pb level-associated effects on decrements in cognitive function and behavior in children, specifically, whether the magnitudes of change have consequences on the health and life-success of individuals. According to the WHO, "Health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity" (WHO, 1948). By this definition, even decrements in health status that are not severe enough to meet diagnostic criteria might be undesirable if they reflect a decrement in the well-being of an individual. Also, decreases in health indices or life-success may not be observable except at the population level. The American Thoracic Society discussed the need to consider the prevalence of exposures in the population and exposure to other risk factors in evaluating whether shifts in the population-level risk are adverse (ATS, 2000). Neurodevelopmental decrements measured in childhood may set affected children on trajectories more prone toward lower educational attainment and financial well-being. Thus, early effects in children may have lifetime consequences. There also may be groups in the population at increased risk of

neurodevelopmental effects from Pb exposure. For example, some evidence points to larger blood Pb-associated decrements in cognitive function in children with lower SES (Ris et al., 2004; Tong et al., 2000; Bellinger et al., 1990), whereas a meta-analysis found a larger effect estimate for studies with higher SES (Schwartz, 1994).

It has been argued that blood Pb-associated decrements in IQ points less than 3 or 4 points are meaningless given that such changes are within the standard error of a single test (i.e., the statistic that defines the range within which the true value of an individual is likely to lie) (Kaufman, 2001). However, this argument incorrectly assumes that conclusions drawn from individual-level data apply to populations. Evidence does not indicate that the standard error is nonrandom, i.e., biased in one direction. Hence, there is no reason to expect that children with higher blood Pb levels systematically test lower than their true IQ value or that children with lower blood Pb levels test higher than their true IQ value. Thus, in a population of children, on a given assessment, some children will test lower than their true value and others will test higher than their true value. In such cases, between-group differences will be measureable on a population basis. Error in the measurement of IQ in an individual will contribute nondifferential error on a population-level and make it more difficult to detect an association if one exists.

The issue of individual-level versus population-level risk also pertains to the implications of the magnitude of decrease in cognitive function or increase in behavioral problems per unit increase in blood Pb level. Although fractional changes in FSIQ, memory, or attention may not be consequential for an individual, they may be consequential on a population level, especially in the two tails of the distribution (Bellinger, 2007, 2004b). For example, interventions that shift the population mean, in a beneficial direction, by an amount that is without clinical consequence for an individual have been shown to produce substantial decreases in the percentage of individuals with values that are clinically significant (Bellinger, 2007, 2004b). In statistical exercises not specific to Pb or analysis of data collected from individuals, Weiss (1990, 1988) predicted that a downward shift of five points in mean IQ, if the amount of dispersion in the distribution remained the same, should be accompanied by a doubling of the numbers of individuals with scores two or more standard deviations below the mean. In this hypothetical model, with a reduction in population mean IQ from 100 to 95, the percentage of individuals predicted to score above 130 (two standard deviations above the mean) decreases from 2.3% to 0.99%. Weiss (1988) stated that the implication of such a loss transcends the current circumscribed definitions of risk. In addition to implications on the loss of intellectual ability, the loss of a few IQ points potentially could result in the loss of academic opportunities in children. For example, schools or programs for the gifted have used IQ cut-offs (e.g., score of 130) to screen or accept applicants. In another hypothetical analysis presented in the 2006 Pb AQCD (U.S. EPA, 2006b), based on a

blood Pb-FSIQ effect estimate of -0.9 points/µg/dL (the median effect estimated for blood Pb levels <10 µg/dL), the fraction of the population with an FSIQ <80 is predicted to more than double from 9% with a blood Pb level of 0 µg/dL to 23% with a blood Pb level of 10 µg/dL (Figure 4-16). The proportion with an FSIQ <70, which often requires community support to live (WHO, 1992), is predicted to increase from a little over 2% with a blood Pb level of 0 µg/dL to about 8% with a blood Pb level of 10 µg/dL [(Figure 4-16) and (U.S. EPA, 2006b)]. These theoretical exercises estimate that for an individual in the low range of the IQ distribution, a Pb-associated decline of a few points might be sufficient to drop that individual into the range associated with increased risk of educational, vocational, and social failure.



Note: The results presented are based on a theoretical analysis of changes in population FSIQ using a concentration-response estimate of -0.9 FSIQ points/µg/dL, which was the median estimate from studies reviewed in the 2006 Pb AQCD for blood Pb levels <10 µg/dL. The analysis estimates increasing proportions of individuals with FSIQ <80 and <70 with increasing blood Pb level.
Source: 2006 Pb AQCD (U.S. EPA, 2006b).

Figure 4-16 Hypothetical effect of increasing blood Pb level on the proportion of the population with FSIQ <80 and <70 points.

The hypothetical predictions presented in Weiss (1990, 1988) and the 2006 Pb AQCD (U.S. EPA, 2006b) have been supported by findings from analyses using data from studies of blood Pb levels and FSIQ decrements in children. Among children in 1st and 2nd grades from towns around Boston, MA, Needleman et al. (1982) found that a 4-point downward shift in the study population mean verbal IQ estimated for tooth Pb levels >24 ppm was associated with a 3-fold increase in the percentage of children with a verbal IQ of <80 and a decrease in the percentage achieving a verbal IQ >125 from 5% to 0%.

The aforementioned hypothetical analyses and those using data collected from individuals that estimate Pb-associated changes in the population IQ distribution assume that the magnitude of change is equal across segments of the IQ distribution. Few studies of Pb and cognitive function have examined whether the effect of Pb varies across the distribution of cognitive function. However, in a recent study of fourth graders across the entire state of North Carolina, Miranda et al. (2009) found that higher blood Pb level measured once in each child between age 9 months and 3 years was associated with larger decreases in fourth grade EOG scores in the lower segment of the EOG distribution. An increase in blood Pb level from 1 to 10 µg/dL was estimated to decrease EOG score by 0.8 points in the 95th percentile of EOG scores but by 2.3 points in the 5th percentile of EOG score (Figure 4-6). These findings by Miranda et al. (2009) based on analysis of a large database representative of fourth graders in North Carolina indicate that a shift in the population mean from increased Pb exposure may increase the proportions of children at the lower end of the cognitive function distribution over that estimated by theoretical analyses.

In summary, the public health significance of evidence demonstrating associations between increases in blood Pb levels and decrements in IQ of children in the range of a few points is supported by hypothetical predictions that a shift in the population mean increases the proportion of individuals in the lower range of cognitive function and decreases the proportion of individuals in the upper range of cognitive function. These changes in the population distribution also were found in children in 1st and 2nd grade in Massachusetts in whom higher tooth Pb level was associated with decrements in IQ (Needleman et al., 1982). Results from a few studies suggested that the public health significance of decrements in cognitive function associated with higher blood Pb levels may be greater in certain groups of children, for example, those in the lower range of cognitive function (Miranda et al., 2009) and those in lower SES groups (Ris et al., 2004; Tong et al., 2000; Bellinger et al., 1990). On a population-level, small Pb-associated decreases in cognitive function could increase the number of individuals at increased risk of educational, vocational, and social failure and decrease the number of individuals with opportunities for academic and later-life success.

4.3.15 Summary and Causal Determination

The collective body of epidemiologic and toxicological evidence integrated across that reviewed in the 2006 Pb AQCD (U.S. EPA, 2006b) and recent studies indicates relationships between Pb exposure and a range of nervous system effects. In children, effects on cognitive function include FSIQ, learning, memory, executive function, and academic performance. Externalizing behaviors in children were evaluated in two groups:

1) attention, impulsivity, hyperactivity, and ADHD; and 2) conduct disorders which included aggression, delinquency, and criminal offenses. Effects on internalizing behaviors include withdrawn behavior and symptoms of depression, fearfulness, and anxiety. Other nervous system effects evaluated in children are auditory function, visual function, and motor function. Relationships for Pb exposure with cognitive function, auditory function, and visual function also were evaluated in adults. Other nervous system effects in adults examined in relation to Pb exposure include those related to psychopathological effects such as symptoms of depression, anxiety, and panic disorder. Additionally, effects on neurodegenerative diseases include Alzheimer's disease, ALS, Parkinson's disease, and essential tremor. The subsequent sections describe the evaluation of evidence for each of these outcome groups with respect to causal relationships with Pb exposure using the framework described in Table II of the Preamble. The application of key evidence, supporting or contradictory, to the causal framework is summarized in Table 4-17.

4.3.15.1 Evidence for Cognitive Function Decrements in Children

A causal relationship between Pb exposure and cognitive function decrements in children is supported by several lines of evidence: 1) findings from prospective studies in diverse populations consistently demonstrating associations of higher blood and tooth Pb levels with lower FSIQ and performance on tests of executive function and academic performance in children ages 4-17 years and young adults ages 18-20 years (Section 4.3.2); 2) coherence with evidence in animals for impairments in learning, memory, and executive function with relevant Pb exposures; and 3) evidence describing modes of action (Table 4-17).

Clear support for Pb-associated cognitive function decrements in children, as described in the 2006 Pb AQCD (U.S. EPA, 2006b), was provided by prospective epidemiologic studies indicating associations of higher earlier childhood (e.g., prenatal maternal or cord, age 2 or 4 year), concurrent (ages 4-10 year), and childhood average blood and tooth Pb levels with lower FSIQ in children ages 4-17 years (Table 4-17 and Section 4.3.2.1). Evidence for such Pb biomarker metrics better characterized the temporal sequence between Pb exposure and decrements in cognitive function than did cross-sectional analyses. Across studies, FSIQ was measured with various instruments (i.e., WISC-R, WISC-III, WPPSI, Stanford-Binet) that were similar in scoring scale and measurement error. Associations were found in most of the prospective studies, conducted in the U.S., Mexico, Europe, and Australia in representative populations, most of which had moderate to high follow-up participation without indication of selective participation among children with higher blood Pb levels and lower cognitive function (Table 4-3). Another

strength of the evidence from prospective studies was the consideration for several potential confounding factors. As indicated in Table 4-3, results from most cohort studies were adjusted for maternal IQ and education, child sex and birth weight, SES, and HOME score. Although not considered as frequently, some studies also found associations with adjustment for parental smoking, birth order, and nutritional factors. The consistency and reproducibility of the blood Pb-FSIQ association in children were demonstrated further in pooled analyses of seven prospective studies (Lanphear et al., 2005; Rothenberg and Rothenberg, 2005) as well as multiple meta-analyses that combined results across various prospective and cross-sectional studies (Pocock et al., 1994; Schwartz, 1994; Needleman and Gatsonis, 1990), with Schwartz (1994) demonstrating the robustness of evidence to potential publication bias. These pooled or meta-analyses combine data that are somewhat heterogeneous in the tests used to assess FSIQ and the ages of blood Pb and FSIQ examined which could be sources of non-differential measurement error. Heterogeneity in the potential confounding factors examined and the method of assessment could produce residual confounding.

Among individual studies, a wide range of blood Pb-FSIQ effect estimates was obtained, which is not unexpected given the wide range of blood Pb levels examined and modeling methods used (i.e., linear, log-linear). The pooled analysis of seven prospective cohorts demonstrated precision of effect estimates by applying a uniform method across populations (Lanphear et al., 2005). A narrow range of estimates was obtained by excluding one study at a time, -2.4 to -2.9 points per log increase in concurrent (ages 4.8-10 years) blood Pb level. Results from several individual studies, in particular prospective studies, indicated a supralinear concentration-response relationship which estimated a greater decrement in cognitive function per unit increase in blood Pb level among children in lower strata of blood Pb levels than children in higher strata of blood Pb levels (Figure 4-15 and Table 4-16).

Among the largest effect estimates were those found in the Boston and Rochester cohorts (Canfield et al., 2003a; Bellinger et al., 1992), which had relatively smaller sample sizes but considered several potential confounding factors as listed above and in Table 4-3, examined lower blood Pb levels than did other prospective studies, and examined cohorts that differed in SES. Thus, their results may be more representative of the effects of Pb exposure on FSIQ. In the Boston cohort, a 1 µg/dL increase in age 2 year blood Pb level was associated with a -1.6 (95% CI: -2.9, -0.2) point change in FSIQ at age 10 years in 48 children with age 2-year blood Pb levels 1-9.3 µg/dL (detection limit not reported) and peak blood Pb levels <10 µg/dL (Bellinger, 2008; Bellinger and Needleman, 2003). In the Rochester cohort, a 1 µg/dL increase in concurrent blood Pb level was associated a -1.8 (95% CI: -3.0, -0.60) change in FSIQ at age 5 years in 101 children with concurrent blood Pb levels 0.5-8.4 µg/dL (detection limit = 1 µg/dL) and peak blood Pb levels

<10 µg/dL (Canfield, 2008; Canfield et al., 2003a). In the pooled analysis, a 1 µg/dL increase in concurrent blood Pb level was associated a -2.9 (95% CI: -5.2, -0.71) change in FSIQ at ages 4.8-10 years in 103 children with a mean concurrent blood Pb level of 3.2 µg/dL and peak blood Pb levels <7.5 µg/dL (Hornung, 2008; Lanphear et al., 2005). Results from the Boston and Rochester cohorts can be considered particularly informative because within each cohort, there was greater homogeneity in the tests used to assess FSIQ, the age of examination, and the methods used to measure potential confounding factors. Further, in Lanphear et al. (2005), 80% of the data for the lower portion of the blood Pb distribution was provided by the Boston and Rochester cohorts. These observations do not identify a threshold in the ranges of blood Pb level examined.

Null or weak associations were limited to a few cohorts, namely, the Cleveland and Sydney cohorts (Greene et al., 1992; Cooney et al., 1991; 1989a, b; Ernhart et al., 1988). The Cleveland and Sydney studies were not outliers with respect to population mean blood Pb levels or the specific confounding factors considered (Table 4-3), and the Cleveland cohort had high prevalence of maternal prenatal substance abuse which may limit the representativeness of results.

Other previous studies estimated smaller magnitude of effects for the blood Pb-FSIQ association in children ages 4-13 years with either linear or log-linear models but examined higher blood Pb levels (means: 7.8-16 µg/dL) and did not analyze the concentration response at the lower range of the study population blood Pb distribution (Table 4-3). Most recent cross-sectional studies supported associations between higher concurrent blood Pb levels and decrements in FSIQ. Some found associations in populations of children ages 6-11 years with lower blood Pb levels than other studies, i.e., means 1.7-5.0 µg/dL (Table 4-17). In exception, among children ages 6-10 years in New England with a mean concurrent blood Pb level 2.2 µg/dL, lower FSIQ was found in the group with blood Pb levels 5-10 µg/dL (Surkan et al., 2007). In many of these studies, the potential influence of higher past Pb exposures cannot be excluded. Although Zailina et al. (2008) had limited consideration for potential confounding, results from most of these studies were adjusted for SES and parental IQ or education. Chiodo et al. (2007) found an association with extensive consideration for potential confounding, including parental caregiving quality and prenatal drug exposure, which was prevalent in the study population.

A causal relationship between Pb exposure and cognitive function decrements in children also is supported by previous prospective studies (several of which contributed to the FSIQ evidence) that found associations of blood or tooth Pb level with decrements in executive function and academic performance in populations ages 4-20 years (Sections 4.3.2.4, 4.3.2.5, and Table 4-17). The bodies of evidence for executive function, and

academic performance are smaller than that for FSIQ but consistently indicate associations with blood or tooth Pb level. Associations with performance on tests of learning and memory were less consistently found across populations (Section 4.3.2.3). In most studies, previous and recent, multiple testing was common; however, the consistent pattern of association observed across the ages of blood Pb level and/or cognitive test examined increases confidence that the evidence is not unduly biased by an increased probability of associations found by chance alone. While recent studies of executive function and academic performance adjusted for SES and parental education and/or cognitive function, few examined potential confounding by parental caregiving quality.

Adding to the evidence for Pb-associated cognitive function decrements in children were recent prospective studies that indicated associations between higher earlier childhood blood Pb level and poorer academic performance in school-aged children (Chandramouli et al., 2009; Miranda et al., 2009). Among 57,678 children in North Carolina, lower fourth grade EOG scores were found in children with age 9-36 month blood Pb levels categorized as 2 µg/dL compared with children with blood Pb levels categorized as 1 µg/dL (detection limit = 1 µg/dL), with adjustment for sex, race, school type, age of blood Pb measurement, parental education, and enrollment in a free lunch program as an indicator of SES (Miranda et al., 2009). Results from this study also indicated a greater incremental association of blood Pb level with decrement in EOG score among children in the lower end of the EOG distribution. Chandramouli et al. (2009) found lower school achievement tests scores in 488 children age 7 years in U.K. in the children with age 30 month blood Pb levels >5 µg/dL. These results were adjusted for several potential confounding factors, including SES, parental education, SES, home facilities score, and family adversity. Recent cross-sectional studies conducted in the U.S. (Krieg et al., 2010; Surkan et al., 2007) found associations of concurrent blood Pb level with decrements in executive function and academic performance in children, including the large analysis of >700 children ages 12-16 years participating in NHANES (Krieg et al., 2010). Cho et al. (2010) did not find an association between concurrent blood Pb level and executive function among children in Korea ages 8-11 years with a mean blood Pb level 1.9 µg/dL.

Several studies found associations of higher prenatal, earlier infancy (e.g., age 1 year), and concurrent blood Pb levels with lower Bayley MDI scores in children ages 2 and 3 years (Table 4-4). Similar to studies of FSIQ, Tellez-Rojo et al. (2006) estimated a larger decrement in age 2 year MDI per unit increase in concurrent blood Pb level for children in Mexico City with blood Pb levels <5 µg/dL compared with children with blood Pb levels 5-10 µg/dL, and >10 µg/dL (Figure 4-15 and Table 4-16). MDI is a well-standardized measure of current infant mental development. However, the test of MDI is not an intelligence test, and MDI scores, particularly before ages 2-3 years, are not

necessarily strongly correlated with later measurements of FSIQ in children with normal development.

A causal relationship between Pb exposure and cognitive function decrements in children is further supported by consistent observations in rodents and monkeys of decrements in learning, associative ability, memory, and executive function with relevant dietary Pb exposures. In particular, coherence was found between evidence in children and animals of Pb-associated decrements in visual-spatial memory, working memory (Section 4.3.2.3) and rule learning and reversal (Section 4.3.2.4). Previous studies in monkeys ages 5-10 years demonstrated impairments in learning, memory, and executive function with dietary Pb exposures during infancy only, lifetime after early infancy, and lifetime from birth that produced blood Pb levels of 15-36 µg/dL, some relevant to this ISA (Rice, 1992b; Rice and Gilbert, 1990a; Rice, 1990; Rice and Gilbert, 1990b; Rice and Karpinski, 1988; Gilbert and Rice, 1987).

Several recent toxicological studies added to the evidence for impaired learning, associative ability, and memory in animals with blood Pb levels, 10-25 µg/dL after gestational-lactational, lactational, or lifetime (with and without gestational) Pb exposure (Cory-Slechta et al., 2010; Niu et al., 2009; Stangle et al., 2007). Together, the prospective epidemiologic and toxicological studies provide evidence for the temporal sequence between Pb exposure and decrements in cognitive function. Additional biological plausibility for Pb-associated cognitive function decrements was provided by toxicological evidence for the effects of Pb on modes of action for cognitive function (Section 4.3.10). Pb has been shown to increase the permeability of the blood-brain barrier and deposit in the target CNS. Pb has been shown to impair neurogenesis, synaptic architecture, and neurite outgrowth. The high activity of these processes during fetal and infant development provides biological plausibility for the effects of childhood Pb exposure on decrements in cognitive function. Cognitive function is mediated by the cortical and subcortical structures of the brain that integrate function in the hippocampus, prefrontal cortex, and nucleus accumbens using dopamine and glutamate as primary neurotransmitters. Experimental studies have shown that Pb induces changes in dopamine and glutamate release in these regions and decreases the magnitude of LTP, which is a major cellular mechanism underlying synaptic plasticity and learning and memory.

With regard to critical lifestages and time periods of Pb exposure, toxicological evidence clearly demonstrates impaired learning and memory in animals exposed to Pb gestationally with or without lactational exposure. This evidence is well supported by knowledge that processes such as neurogenesis, synaptogenesis, and synaptic pruning are very active during this developmental period. However, evidence in monkeys also indicates impaired cognitive function at ages 5-8 years with Pb exposure starting after

early infancy (Rice, 1992b; Rice and Gilbert, 1990a; Rice, 1990; Rice and Gilbert, 1990b). Epidemiologic studies also found cognitive function decrements associated with blood Pb levels measured during various lifestages and time periods. Distinguishing among the effects of Pb exposures at different time periods is difficult in epidemiologic studies due to the high correlations commonly found among blood Pb levels within children over time. Studies of young children ages 6 months to 3 years found lower MDI in association with higher prenatal maternal or cord and postnatal child blood Pb levels; some found larger magnitude decrements for prenatal blood Pb (Hu et al., 2006; Gomaa et al., 2002). However, in older children, ages 4-17 years, in whom cognitive function is more stable and reliably measured, larger decrements in cognitive function were found in association with increases in postnatal blood Pb levels (concurrent, earlier childhood, and cumulative average) than increases in prenatal blood Pb levels. There was no clear indication of an individual critical lifestage, timing, or duration of Pb exposure associated with cognitive function decrements in children. Because of the contribution of bone Pb levels to concurrent blood Pb levels in children, associations with concurrent blood Pb levels may reflect an effect of past and/or recent Pb exposures.

The consideration for potential confounding varied among studies. Most studies adjusted for SES-related factors such as the Hollingshead Index, household income, and/or parental education. Several, in particular the prospective studies, additionally adjusted for parental cognitive function and caregiving quality mostly evaluated as HOME score. A few studies considered nutritional factors. Not many recent studies considered potential confounding by parental caregiving quality or nutritional factors. Controlling for SES is difficult as it can be highly correlated with Pb exposure and there is no single measure that fully represents SES. Residual confounding also is possible by factors not considered or measured inadequately. The combination of evidence from prospective studies that considered several well-characterized potential confounding factors plus evidence that Pb exposure induces impairments in cognitive function in animals, in particular, for similar constructs as those associated with Pb biomarkers in children, increase confidence that the associations observed between blood Pb levels and cognitive function in children represent a relationship with Pb exposure.

In conclusion, multiple prospective studies conducted in diverse populations consistently demonstrate associations of higher blood and tooth Pb levels with lower FSIQ, executive function, and academic performance and achievement in children. Most studies examined representative populations and had moderate to high follow-up participation without strong indication of selection bias. Associations between blood Pb level and cognitive function decrements were found with adjustment for several potential confounding factors, most commonly, SES, parental IQ, parental education, and parental caregiving quality. Among studies that examined early childhood blood Pb levels (i.e., age < 3

years), considered peak blood Pb levels in their analysis (i.e., peak < 10 µg/dL), or examined concurrent blood Pb levels in young children (i.e., age 4 years), blood Pb-associated decrements in cognitive function were found in populations of children ages 4-11 years with mean or group blood Pb levels in the range of 2 to 8 µg/dL. Neither epidemiologic nor toxicological evidence has identified an individual critical lifestage or time period of Pb exposure within childhood that is associated with cognitive function decrements. Several epidemiologic studies found a supralinear concentration-response relationship. Examination of children with blood Pb levels in the range <1 (at or below detection limits) to 10 µg/dL, with consideration of early or peak childhood blood Pb levels, has not identified a threshold for cognitive function decrements. Evidence in children was clearly supported by observations of Pb-induced impairments in learning, memory, and executive function in rodents and monkeys. Several studies in animals indicated cognitive impairments with prenatal, lactational, and lifetime (with or without prenatal) Pb exposures that resulted in blood Pb levels of 10-25 µg/dL. The mode of action for Pb-associated cognitive function decrements is supported by observations of Pb-induced impairments in neurogenesis, synaptogenesis and synaptic pruning, LTP, and neurotransmitter function in the hippocampus, prefrontal cortex, and nucleus accumbens. The associations consistently found for FSIQ and other measures of cognitive function in prospective studies of children with adjustment for SES, parental education, and parental caregiving quality and the biological plausibility provided by evidence in animals for impairments in learning, memory, and executive function with relevant Pb exposures and evidence describing modes of action are sufficient to conclude that there is a causal relationship between Pb exposure and decrements in cognitive function in children.

4.3.15.2 Evidence for Externalizing Behaviors: Attention, Impulsivity, and Hyperactivity in Children

Attention, impulsivity, and hyperactivity are evaluated together because they are included within the attention deficit hyperactivity disorder domain of externalizing behaviors (as reviewed in Whitcomb and Merrell, 2012). Although examined less extensively than cognitive function, a causal relationship between Pb exposure and attention decrements, impulsivity, and hyperactivity in children is supported by multiple lines of evidence: 1) findings from prospective studies in diverse populations for associations with blood or tooth Pb levels; 2) coherence with evidence in animals with relevant Pb exposures and; 3) evidence for plausible modes of action (Table 4-17). As described below, this conclusion is based on evidence primary for attention decrements assessed with neuropsychological testing or rated by parents or teachers and supporting evidence for impulsivity and hyperactivity. The few studies showing blood Pb-associated increases in ADHD prevalence are not a major consideration because of the cross-sectional or case-control

design of studies and inconsistent consideration for potential confounding by factors such as SES, parental education, or parental caregiving quality (Section 4.3.3.1).

Prospective studies provided key evidence for associations of childhood blood and tooth Pb levels with attention decrements or hyperactivity in children ages 7-17 years and young adults ages 19-20 years. Not all results were statistically significant, but results within and across studies mostly showed a pattern of attention decrements or hyperactivity with higher blood or tooth Pb level. The pattern of association observed within studies increases confidence that the evidence is not unduly biased by the increased probability of finding associations by chance alone. The results from prospective studies better characterized the temporal sequence between Pb exposure and measured behaviors than did cross-sectional studies. Associations were found in populations in the U.S., U.K., Australia, and New Zealand (Table 4-17). Most studies had population-based recruitment from prenatal clinics, hospitals at birth, or schools and had moderate to high participation. A few prospective studies had greater loss-to-follow-up in certain groups, for example, those with lower SES, earlier FSIQ, or HOME score. This potential selection bias may have reduced the generalizability of findings to the original study population, but there was not a strong indication that participation was biased to those with higher blood Pb levels and greater behavioral decrements.

Key evidence provided by prospective studies supported associations of blood and tooth Pb levels with attention decrements as assessed using neuropsychological tests or parent or teacher ratings using widely-used structured questionnaires (Section 4.3.3.1). Thus, the collective evidence does not appear to be unduly influenced by biased reporting of such behaviors by parents of children with higher blood or tooth Pb levels. Prospective studies that examined attention with the CPT found associations of higher prenatal or earlier childhood (age 6 year, age 3-60 month) blood Pb levels or tooth (from 1st/2nd grade) Pb levels with increases in commission, omission, or reaction time errors in adolescents ages 15-17 years in Cincinnati (Ris et al., 2004) and young adults 19-20 years in Chelsea/Somerville, MA (Bellinger et al., 1994a). Results from prospective studies also indicated associations of lifetime (to ages 11-13 years) average blood Pb levels and tooth (from ages 6-8 years) Pb levels with lower parental and teacher ratings of attention in children ages 8-13 years in Australia, New Zealand, and Boston, MA (Burns et al., 1999; Fergusson et al., 1993; Leviton et al., 1993). A recent prospective study in the U.K. found that children ages 7-8 years with age 30 month blood Pb levels > 10 µg/dL had higher teacher ratings of hyperactivity but not selective attention compared with children with blood Pb levels 0-2 µg/dL (Chandramouli et al., 2009). Results from children participating in a chelation trial suggested a blood Pb-hyperactivity relationship that was mediated through an association with IQ (Chen et al., 2007). Previous findings for hyperactivity were limited to cross-sectional and case-control studies (Section 4.3.3.1).

Lifetime average blood Pb levels were not associated with ratings of attention in younger children ages 4-5 years in Yugoslavia (Wasserman et al., 2001). However, attention may be less reliably rated in such young children.

Attention decrements were associated with biomarkers of Pb exposure representing various lifestages and time periods. Prospective studies did not examine a detailed Pb biomarker history, and results do not identify an individual critical lifestage, time period, or duration of Pb exposure associated with attention decrements. Associations in prospective studies for attention decrements with tooth Pb level, early childhood average and lifetime average blood Pb levels point to an effect of cumulative Pb exposure. Prospective studies did not examine biomarkers Pb exposure measured closer to the time of behavioral assessment. Across prospective studies, attention decrements were associated with blood Pb levels that had population means of 6.8 and 8.3 µg/dL for prenatal (maternal or cord) blood, 13.4 for age 3-60 month average blood, and 14 µg/dL for lifetime average blood. In Yugoslavia study with null results, the mean lifetime average blood Pb level was 7.2 µg/dL, similar to those associated with attention decrements in some of the other prospective studies.

A causal relationship between Pb exposure and attention, hyperactivity, and impulsivity is supported further by the consideration for several potential confounding factors in prospective studies. Although the specific factors varied by study, prospective studies adjusted for factors such as SES, parental IQ, maternal education, self drug use, prenatal drug and alcohol exposure, birth outcomes, and parental caregiving quality. Parental caregiving quality has been speculated as a means by which parental psychopathology may influence child Pb exposure. However, a clear relationship between parental psychopathology and poorer parenting behavior has not been established (as reviewed in Johnston et al., 2012), and adjustment for parental psychopathology may adjust for Pb exposure itself. At present, there is not evidence to support consideration of parental psychopathology as a direct confounding factor, i.e., contributing to a spurious association with Pb biomarkers when there is none. However, if there is a noncausal correlation between parental psychopathology and Pb exposure and it is mediated by parental caregiving quality, then adjustment for parental caregiving quality also may help address potential confounding by parental psychopathology.

Consistent with previous prospective studies, cross-sectional studies found associations of higher concurrent blood or tooth Pb level with attention decrements, impulsivity, and hyperactivity in children ages 3-16 years (Table 4-17). Because of the greater uncertainty about the potential for reverse causation, cross-sectional results are a lesser consideration in conclusions. The cross-sectional studies included a mix of previous and recent studies, a mix of preschool-aged and school-aged children, and a wide range of population mean

blood Pb levels (1-12 µg/dL). Among the studies examining populations in the lower part of this blood Pb distribution (means: 5-6.5 µg/dL for ages 4-7.5 years) and with more extensive consideration for potential confounding, most found associations with attention decrements, hyperactivity, and/or impulsivity (Plusquellec et al., 2010; Chiodo et al., 2007; Chiodo et al., 2004). All of these studies adjusted for or considered potential confounding by SES as well as parental IQ, education, and caregiving quality. Among children ages 4-6 years in whom behavior may be less reliably measured, concurrent blood Pb level was associated with higher examiner ratings of attention decrements, impulsivity, and hyperactivity in Inuit children ages 5-6 years in Canada (Plusquellec et al., 2010) but not in the Rochester cohort at age 4.5 years (Canfield et al., 2003b). Also, in the Rochester cohort, the association with attention was attenuated only after adjustment for color/shape knowledge and child IQ. These inter-related outcomes may share some variance. Among older children (age 7-7.5 years) in Detroit, MI, higher concurrent blood Pb level (means: 5; 5.4 µg/dL) was associated with higher examiner or teacher ratings of attention decrements and hyperactivity (Chiodo et al., 2007; Chiodo et al., 2004). These studies also found associations with attention decrements as assessed by the CPT, although not strongly with all examined indices of the CPT. These populations had high prevalence of prenatal alcohol or drug exposure, but results did not indicate confounding by these other exposures. Nonetheless, the presence of these other exposures may limit the generalizability of results. While higher-quality cross-sectional evidence in populations with mean blood Pb levels 5-6.5 µg/dL show associations of blood Pb level with attention decrements, impulsivity, and hyperactivity, there are limitations related to young age of subjects, generalizability of the study population, or potential contribution of higher blood Pb levels earlier in childhood.

Other cross-sectional studies examined even lower concurrent blood Pb levels (means: 1-3.7 µg/dL) and found associations with attention decrements, impulsivity, hyperactivity, or a composite index of ADHD-related behaviors in children ages 8-17 years (Cho et al., 2010; Nicolescu et al., 2010; Froehlich et al., 2009; Nigg et al., 2008). These associations were found with parental or teacher ratings, with attention measured by the CPT, and impulsivity measured with response inhibition tests. However, results have weaker implications because of less extensive consideration for potential confounding, case-control study design, and uncertainty regarding the influence of higher blood Pb levels earlier in childhood. Studies varied in adjusting results for SES and parental education, and none considered parental caregiving quality. Most studies of populations with relatively high concurrent blood Pb levels (means 11-12 µg/dL for ages 3-11 years) also found that higher blood Pb levels were associated with higher parent or teacher ratings of attention decrements and hyperactivity (Roy et al., 2009; Kordas et al., 2007; Silva et al., 1988). Most cross-sectional studies of higher blood Pb levels found associations with adjustment for SES, child age, sex, family structure, and parental

education, but most did not examine parental caregiving quality. With the exception of findings from the Rochester cohort (Canfield et al., 2003b), studies generally found associations between blood Pb level and attention decrements, impulsivity, and hyperactivity with adjustment for child IQ or other measure of cognitive function (Cho et al., 2010; Chandramouli et al., 2009; Nigg et al., 2008; Silva et al., 1988). These findings add support for higher Pb exposures having effects on attention, impulsivity, and hyperactivity, independent of effects on cognitive function.

Further support for a causal relationship between Pb exposure and attention, impulsivity, and hyperactivity is provided by observations of greater impulsivity in animals with relevant dietary Pb exposures. Evidence in rodents and monkeys demonstrates Pb-induced impaired response inhibition by decreased interresponse times and increased overall response rates in FI reinforcement schedules (Table 4-7) (Rossi-George et al., 2011) , slower acquisition of a DRL schedule (Rice and Gilbert, 1985), and response perseveration on tests of discrimination reversal learning and FR/waiting for reward (Brockel and Cory-Slechta, 1999b, 1998; Gilbert and Rice, 1987). In particular, coherence is found with observations in children of associations between blood Pb levels and poorer performance on the stop signal task, which also measures response inhibition. Impulsivity in rodents and monkeys was found with gestational, post-weaning and lifetime (from gestation or birth) dietary Pb exposures that resulted in blood Pb levels 11-29 µg/dL (Table 4-17 and Section 4.3.3.1). In animals, the effects of Pb exposure on sustained attention were inconsistent as assessed with signal detection or discrimination reversal tests with distracting stimuli (Table 4-17 and Section 4.3.3.1). A recent study did not clearly indicate an effect of Pb on activity in male mice as an increase was found only with amphetamine co-treatment (Leasure et al., 2008); thus, the findings may not be directly comparable to observations of Pb-associated increases in hyperactivity in children.

Additional support for a causal relationship between Pb exposure and attention decrements, impulsivity, and hyperactivity is provided by evidence describing modes of action. These behaviors have been linked with changes in the prefrontal cerebral cortex, cerebellum, and hippocampus, and Pb exposure has been found to affect development and neuronal processes in these regions. For example, Pb has been found to affect dopaminergic neurons of the frontal cortex and striatum of the brain by altering dopamine release and receptor density. Other lines of evidence supporting the mode of action for the effects of Pb exposure on attention, impulsivity, and hyperactivity include Pb-induced changes in neurogenesis, synapse formation, and synaptic plasticity.

In conclusion, although examined less extensively than cognitive function, several prospective studies demonstrated associations of blood or tooth Pb levels measured years

before outcomes with attention decrements and hyperactivity in children 7-17 years and young adults ages 19-20 years as assessed using objective neuropsychological tests and rated by parents and teachers. Most of these prospective studies examined representative populations without indication of participation conditional on blood Pb levels and behavior. The results from prospective studies were adjusted for potential confounding by SES as well as parental education and caregiving quality, with some studies also considering parental cognitive function, birth outcomes, substance abuse, and nutritional factors. In prospective studies, blood Pb-associated attention decrements and hyperactivity were found in populations with prenatal (maternal or cord), age 3-60 month average, age 6 year, or lifetime average (to age 11-13 years) mean blood Pb levels of 7 to 14 µg/dL and in groups with age 30 month blood Pb levels >10 µg/dL. Most well-conducted cross-sectional studies that examined several potential confounding factors found associations of attention decrements, impulsivity, and hyperactivity in children ages 5-7.5 years with concurrent blood Pb levels with means of 5-5.4 µg/dL but cannot establish temporality or exclude the influence of higher blood Pb levels earlier in childhood. Biological plausibility for observations in children is provided by several findings in animals for increases in impulsivity or impaired response inhibition with relevant post-weaning and lifetime Pb exposures that resulted in blood Pb levels of 11 to 29 µg/dL. The mode of action for Pb-associated attention decrements, impulsivity, and hyperactivity is supported by observations of Pb-induced impairments in neurogenesis, synaptic pruning, and dopamine transmission in the prefrontal cerebral cortex, cerebellum, and hippocampus. The consistency of epidemiologic evidence for attention decrements and hyperactivity from prospective studies and the biological plausibility provided by evidence for Pb-induced impulsivity in animals and for underlying modes of action is sufficient to conclude that there is a causal relationship between Pb exposure and effects on attention, impulsivity, and hyperactivity in children.

4.3.15.3 Evidence for Externalizing Behaviors: Conduct Disorders in Children and Young Adults

There are two domains of conduct disorders: undersocialized aggressive conduct disorder and socialized aggressive conduct disorder (as reviewed in Whitcomb and Merrell, 2012). In this evaluation of a relationship with Pb exposure, the two domains are combined because it is difficult to differentiate between these two domains in the available epidemiologic studies. Criminal offenses are included in the evaluation because they can be predicted by earlier conduct disorders (Soderstrom et al., 2004; Babinski et al., 1999; Pajer, 1998). A causal relationship is likely to exist between Pb exposure and conduct disorders in children and young adults based primarily on epidemiologic evidence (Section 4.3.3.2). Key evidence is provided by most previous and recent prospective

studies that found associations of blood Pb levels with higher parent and teacher ratings of delinquent, aggression, and antisocial behavior in children ages 8-17 years from diverse locations and SES (i.e., U.K., Cincinnati, Port Pirie, Australia) (Table 4-12 and Table 4-17). Associations were found with lifetime average blood Pb levels in boys ages 11-13 years in Port Pirie, Australia (Burns et al., 1999), with age 30 month blood Pb levels in children ages 8 years in the U.K. (Chandramouli et al., 2009), and age 0-6 year average blood Pb levels in the Cincinnati cohort at age 15-17 years (Dietrich et al., 2001). Additional support was provided recent prospective studies finding associations of higher earlier childhood blood (age 6 years) or tooth (from ages 6-8 years) Pb levels with criminal offenses in young adults in Cincinnati and Christchurch, New Zealand, ages 19-24 years, as assessed through government records (Fergusson et al., 2008; Wright et al., 2008). In the Cincinnati cohort, a 1 µg/dL increase in age 6 year blood Pb level was associated with an increased risk of criminal arrests at age 19-24 years with an RR of 1.05 (95% CI: 1.01, 1.09). The moderate to high follow-up participation and associations found with parent and teacher ratings of behaviors related to conduct disorders do not provide strong evidence for biased participation or reporting of behaviors for children with higher blood Pb levels. Studies of criminal offenses and ratings of behaviors related to conduct disorders found associations with adjustment for several potential confounding factors such as SES, exposure to smoking, drugs, or alcohol, and parental caregiving quality. Adjustment for parental caregiving quality may also address potential confounding by parental psychopathology. Parental caregiving quality has been speculated as a means by which parental psychopathology may influence child Pb exposure. However, a clear relationship between parental psychopathology and poorer parenting behavior has not been established (as reviewed in Johnston et al., 2012). There is not evidence to support consideration of parental psychopathology as a direct confounding factor.

Supporting evidence was provided by cross-sectional studies, including the large analysis of 2,867 children ages 8-15 years participating in NHANES, which found that compared with children with concurrent blood Pb levels 0.2-0.7 µg/dL (detection limit=0.3 µg/dL), children with concurrent blood Pb levels 0.8-1.0 µg/dL had higher odds of conduct disorder as assessed by parents with adjustment for age, sex, race, poverty income ratio, and smoking exposure (Braun et al., 2008). Chiodo et al. (2007) found an association between concurrent blood Pb level and teacher ratings of social problems and delinquent behavior in children ages 7 years in Detroit, MI after extensive consideration for potential confounding factors including parental caregiving quality as well as prenatal drug exposure, which was prevalent in the population. Further supporting the consistency of association between blood Pb levels and behaviors related to conduct disorders in children, a recent meta-analysis found that evidence was robust to heterogeneity in study

design, definition and assessment method of conduct disorders, potential confounding variables examined, and range of blood Pb levels (Marcus et al., 2010).

Associations of measures of conduct disorders in children and young adults with earlier childhood, earlier childhood average, and lifetime average blood Pb levels, tooth Pb levels, and concurrent bone Pb levels point to the effects of early childhood or cumulative Pb exposures. Most prospective studies did not analyze Pb biomarker levels at multiple lifestages or time periods and thus did not provide information on potential associations with more recent blood Pb levels or differences in association among Pb biomarkers at various time periods. With respect to blood Pb levels, an association with criminal offenses was found in young adults ages 19-24 years with a mean age 6 year blood Pb level of 6.8 µg/dL, and associations with behaviors related to conduct disorders were found in children age 8 years with age 30 month blood Pb levels >10 µg/dL, boys ages 11-13 years with a mean lifetime average blood Pb level of 14 µg/dL, and children ages 15-17 years with a median age 6 year blood Pb level of 12 µg/dL (Table 4-17). Cross-sectional studies found associations with concurrent blood Pb level that were lower: 0-8-1.0 µg/dL for ages 8-15 years and a mean of 5 µg/dL for children age 7 years. However, the influence by higher past Pb exposures cannot be excluded.

Despite evidence from several high-quality epidemiologic studies that have examined several potentially important confounding factors, an important uncertainty remains. Evidence of Pb-induced aggression in animals was inconsistent, with increases in aggression found in some studies of adult animals with gestational plus lifetime Pb exposure but not juvenile animals (Section 4.3.3.2). The lack of biological plausibility from animal evidence and the relatively small body of epidemiologic evidence produces uncertainty regarding the effects of Pb exposure independent from other factors.

In conclusion, the few prospective studies consistently indicate that earlier childhood (age 30 months, 6 years) or lifetime average (to age 11-13 years) blood Pb levels or tooth (from ages 6-8 years) Pb levels are associated with criminal offenses in young adults ages 19-24 years and with higher parent and teacher ratings of behaviors related to conduct disorders in children ages 8-17 years. Pb-associated increases in conduct disorders were indicated in populations with mean blood Pb levels 7-14 µg/dL. Supporting evidence is provided by a few cross-sectional studies and a meta-analysis of prospective and cross-sectional studies. Associations with lower blood Pb levels, as found in the cross-sectional studies, may have been influenced by higher earlier Pb exposures. Overall, associations were found without indication of strong selection bias and with adjustment for SES, family functioning, parental education, IQ, and caregiving quality, and maternal smoking and substance abuse. Evidence for Pb-induced aggression in animals is mixed. The consistent epidemiologic evidence from prospective and cross-sectional studies for

criminal offenses and measures of conduct disorders, but uncertainty due to lack of clear evidence for aggression in animals, is sufficient to conclude that a causal relationship is likely to exist between Pb exposure and conduct disorders in children and young adults.

4.3.15.4 Evidence for Internalizing Behaviors in Children

A causal relationship is likely to exist between Pb exposure in children and internalizing behaviors, including withdrawn behavior and symptoms of depression, fearfulness, and anxiety based on evidence from a few epidemiologic studies and a few animal toxicological studies. Internalizing behaviors have been examined to a lesser extent than cognitive function or externalizing behaviors. However, supporting evidence is provided by a few previous prospective studies that found associations of higher lifetime average blood or tooth Pb levels with higher parent and teacher ratings of withdrawn behavior or symptoms of anxiety and depression in school-aged children, 8-13 years (Burns et al., 1999; Bellinger et al., 1994b) (Section 4.3.4.1, Table 4-17). These prospective studies followed children from birth and had moderate follow-up participation to later childhood. Participation was not conditional on early childhood blood Pb levels, and associations were found with both parent and teacher ratings of internalizing behaviors, reducing the likelihood of undue influence of biased participation and behavioral ratings by parents of children with higher Pb exposures. Internalizing behaviors were assessed with widely-used structured questionnaires such as the Child Behavior Checklist but not assessed as clinically-diagnosed conditions such as depression.

The analysis of the Port Pirie, Australia cohort had the most extensive consideration of potential confounding. Among only the 163 girls, ages 11-13 years, Burns et al. (1999) found that a 1 µg/dL increase in lifetime average blood Pb level in the interval 13.3-14.9 µg/dL (10th-90th percentiles) was associated with an increased odds of an anxious/depressed parental rating above the median of 1.07 (95% CI: 1.01, 1.14) with the adjustment for several SES-related variables and factors related to parental caregiving including HOME score, family functioning score, and current maternal psychopathology. In a Boston-area cohort, Pb level in deciduous teeth (from age 6 years) was associated with a higher teacher rating of a composite of anxious and social withdrawn behaviors in children ages 8 years with adjustment for receiving public assistance at birth and maternal education (Bellinger et al., 1994b). Tooth Pb level was not associated with internalizing behaviors in another Boston-area cohort at age 19-20 years (Bellinger et al., 1994a). In the Yugoslavia cohort, higher lifetime average blood Pb levels (mean: 7.2 µg/dL) were associated with higher maternal ratings of anxious-depressed and withdrawn behaviors in 191 children ages 4-5 years, with stronger associations found with delinquent behaviors (Wasserman et al., 2001). Behavior ratings may be less reliable in

these younger children. With respect to critical lifestages and durations of Pb exposure, evidence from prospective studies for associations with tooth Pb levels and lifetime average blood Pb levels indicates an effect of cumulative Pb exposure on increasing internalizing behaviors in children. While these studies adjusted for SES, birth outcomes, and parental education, few considered parental caregiving quality. Thus, there is uncertainty regarding potential confounding by parental caregiving quality.

Cross-sectional studies, including several recent studies, indicated associations between concurrent blood Pb levels and internalizing behaviors in children ages 3-16 years. Among the cross-sectional studies with more extensive consideration for potential confounding and examination of relatively low concurrent blood Pb levels, i.e., means ~5 µg/dL, results were inconsistent (Table 4-17). Previously, Chiodo et al. (2004) found that among children age 7 years in Detroit, MI, HOME score, SES, maternal education, and prenatal alcohol and drug exposure did not influence associations between blood Pb level (mean: 5 µg/dL) and internalizing behaviors. While the high prevalence of prenatal alcohol and drug exposure may not affect internal validity of results, they may limit the generalizability of findings. A lack of association between concurrent blood Pb level and internalizing behaviors was reported in Inuit children age 5 years in Quebec, Canada with a mean blood Pb level of 5.4 µg/dL with consideration of potential confounding by parental caregiving quality, caregiver education and IQ, blood Hg and PCB levels, and prenatal smoking and alcohol exposure (Plusquellec et al., 2010).

Some of the uncertainty in the small epidemiologic evidence base regarding a relationship between Pb exposure and internalizing behaviors is addressed by the biological plausibility provided by the coherence with evidence from a few studies that relevant Pb exposures resulted in depression-like behavior or emotionality in rodents, i.e., dietary lactational Pb exposure producing blood Pb levels of 13 and 17 µg/dL (Beaudin et al., 2007; Dyatlov and Lawrence, 2002). Other studies found Pb-induced increases in emotionality, depression-like behavior, and tactile defensiveness in animals with blood Pb levels >30 µg/dL after gestational/lactational, lactational, or other early postnatal Pb exposure (Section 4.3.4.2). Biological plausibility for Pb-associated increases in internalizing behaviors also is provided by evidence that describes mode of action, including Pb-induced changes in the HPA axis (Section 4.3.2.3) and dopaminergic and GABAergic systems ((Sections 4.2.2.2, 4.3.10.4, and 4.3.10.8), which are found to affect mood and emotional state.

In conclusion, prospective studies in a few populations demonstrate associations of higher lifetime average blood (mean: ~14 µg/dL, age 11-13 years) or childhood tooth (from ages 6-8 years) Pb levels with higher parent and teacher ratings of internalizing behaviors such as withdrawn behavior and symptoms of depression, fearfulness, and

anxiety in children ages 8-13 years. The lack of selective participation by blood Pb level and associations found with parental and teacher behavioral ratings do not provide strong indication of selection bias. The few cross-sectional studies in populations ages 5 and 7 years with mean concurrent blood Pb levels of 5 µg/dL produced inconsistent findings. Pb-associated increases in internalizing behaviors were found with adjustment for maternal education and SES-related variables, and in a few studies, consideration for parental caregiving quality. Some uncertainty in the epidemiologic evidence for the effects of Pb on internalizing behaviors is addressed by the biological plausibility provided by a few findings in animals with dietary lactational Pb exposure producing blood Pb levels relevant to this ISA. Additional toxicological evidence supports modes of action, including Pb-induced changes in the HPA axis and dopaminergic and GABAergic systems. The evidence from prospective studies in a few populations of children and the coherence with evidence from a few animal studies with relevant Pb exposures and with evidence for mode of action but some uncertainty related to potential confounding by parental caregiving quality in studies of children is sufficient to conclude that a causal relationship is likely to exist between Pb exposure and internalizing behaviors in children.

4.3.15.5 Evidence for Auditory Function Decrements in Children

A causal relationship is likely to exist between Pb exposure and auditory function decrements in children based on primarily on epidemiologic evidence provided by a previous prospective study indicating associations of poorer auditory processing with infancy, childhood, and lifetime average blood Pb levels in 215 children age 5 years (Dietrich et al., 1992) and previous large (n = >3,000) cross-sectional NHANES and HHANES studies showing associations of increasing hearing thresholds with concurrent blood Pb levels in children ages 4-19 years (Schwartz and Otto, 1991, 1987). The high follow-up participation from birth in the Cincinnati cohort and the examination of multiple exposures and outcomes in NHANES and HHANES reduce the likelihood of biased participation by children specifically with high blood Pb levels.

The epidemiologic evidence in children is strengthened by the consideration for several potential confounding factors. In the Cincinnati cohort at age 5 years, higher prenatal maternal, neonatal (age 10 day, mean: 4.8 µg/dL), and various postnatal blood Pb levels were associated with poorer auditory processing with adjustment for SES, HOME score, several birth outcomes, and maternal alcohol consumption and with consideration for factors such as maternal smoking and child health (Dietrich et al., 1992). In NHANES and HHANES, higher concurrent blood Pb levels (median: 8 µg/dL) were associated with

increased hearing thresholds with adjustment for age, sex, race, family income, parental education, and nutritional factors (Schwartz and Otto, 1991, 1987).

Additional support for a relationship between Pb exposure and auditory function decrements in children is provided by evidence supporting modes of action. A previous prospective study in children in Mexico City (n = 100-113) found associations of prenatal maternal (range: 1-8 µg/dL) and age 1 and 4 year blood Pb levels (age 2 year mean: 10.8 µg/dL) with lower auditory evoked potentials (Rothenberg et al., 2000). Increased hearing thresholds or latencies in brainstem auditory evoked potentials were found in adult monkeys (ages 8-13 years) with lifetime Pb exposure beginning at gestation or birth (Rice, 1997; Lilienthal and Winneke, 1996). Smaller effects were induced by Pb exposure during gestation only or lactation only (to age 5 months) (Laughlin et al., 2009). In animals, auditory effects were examined with higher Pb exposures than those relevant to this ISA (i.e., blood Pb levels 33-150 µg/dL at peak or testing); thus, it is difficult to assess coherence with observations in children.

In conclusion, evidence from a prospective study and cross-sectional studies in a few U.S. populations of children indicates associations of higher blood Pb level with increases in hearing thresholds and decreases auditory processing or auditory evoked potentials with adjustment for potential confounding by SES in most studies and by child health and nutritional factors in some studies. The high participation rates, particularly in the prospective study with follow-up from birth, reduce the likelihood of biased participation by children with higher blood Pb levels. Across studies, associations were found with blood Pb levels measured at various time periods, including prenatal maternal, neonatal (10 day, mean 4.8 µg/dL), lifetime average (to age 5 years), and concurrent (ages 4-19 years) blood Pb levels (median 8 µg/dL). Evidence for Pb-associated increases in hearing thresholds or latencies of auditory evoked potentials was found in adult monkeys with lifetime dietary Pb exposure. However, these effects in adult animals were found with higher peak or concurrent blood Pb levels (i.e., 33-150 µg/dL) than those relevant to this ISA; thus, the biological plausibility for epidemiologic observations is unclear. The evidence in children, particularly that from a prospective study, but uncertainties related to effects on auditory function in juvenile animals with relevant Pb exposures, is sufficient to conclude that a causal relationship is likely to exist between Pb exposure and decrements in auditory function in children.

4.3.15.6 Evidence for Visual Function Decrements in Children

The effects of relevant Pb exposures on visual function related to CNS changes specifically in children have been examined in few toxicological and epidemiologic studies. An epidemiologic study in children (Rothenberg et al., 2002b) and toxicological studies in adult rats found Pb-associated supernormal ERGs (Fox et al., 2008). However, the relevance of supernormal ERGs to visual function is unclear. Adult animal studies also showed subnormal ERGs depending on the timing of Pb exposure and blood Pb level. Toxicological studies also have characterized potential mechanisms leading to retinal changes including alterations in morphology and cell architecture, signaling, ATPase activity, neurotransmitter levels, cell proliferation, and retinal cell apoptosis. A study of infant monkeys did not find infancy Pb exposure to affect on visual acuity (Laughlin et al., 2008) and used higher exposures than those relevant to this ISA (blood Pb levels 35-40 µg/dL). Because the available epidemiologic and toxicological evidence is of insufficient quantity, quality, and consistency, the evidence is inadequate to determine that a causal relationship exists between Pb exposure and visual function in children.

4.3.15.7 Evidence for Motor Function Decrements in Children

A causal relationship is likely to exist between Pb exposure and motor function decrements in children based mostly on evidence from previous prospective epidemiologic studies with some supporting toxicological evidence. In the Cincinnati cohort, higher neonatal, concurrent, and lifetime average blood Pb levels (means: 4.8, 10, and 12 µg/dL, respectively) were associated with poorer fine and gross motor function in children at age 6 years (Bhattacharya et al., 1995; Dietrich et al., 1993a), and higher age 0-5 year average (mean: 12 µg/dL) and 78 month blood Pb levels were associated with poorer fine and gross motor function in children at ages 15-17 years (Bhattacharya et al., 2006; Ris et al., 2004). In the Yugoslavian cohort, higher lifetime average blood Pb level was associated with decrements in fine but not gross motor function at age 4.5 years (Wasserman et al., 2000b). In these studies, follow-up participation was not conditional on blood Pb levels and motor function, reducing the likelihood of selection bias affecting results. Motor function was assessed using varied but widely-used, structured tests. The evidence from the Cincinnati and Yugoslavia cohorts is substantiated by the consideration of several potential confounding factors such as SES, parental caregiving quality, child health, and in adolescents, marijuana use (Ris et al., 2004). In the prospective studies, the population mean blood Pb levels were higher than those of most of the current U.S. population. Recent cross-sectional studies examining concurrent blood Pb levels that were lower, means 2-5 µg/dL, produced mixed results. Higher concurrent

blood Pb level was associated with poorer motor function with adjustment for several potential confounding factors in 110 Inuit children ages 4-6 years living in subsistence fishing communities (Despres et al., 2005) but not in a more representative population of 534 children ages 6-10 years in New England (Surkan et al., 2007) or in children ages 7-16 years in Korea (Min et al., 2007).

Epidemiologic evidence is supported by observations of poorer balance as assessed with the rotarod test in male (not female) mice with relevant blood Pb levels, i.e., 10 µg/dL after dietary Pb exposure from gestation to PND10 (Leasure et al., 2008). Other toxicological studies produced mixed results for effects on endurance, coordination, and balance (Section 4.3.7) but are less relevant to this ISA because of the higher Pb exposure concentrations examined, i.e., those producing blood Pb levels >30 µg/dL.

In conclusion, evidence from prospective studies in a few populations indicates associations of decrements in fine and gross motor function with higher neonatal, concurrent, and lifetime average blood Pb levels in children ages 4.5-6 years and with higher earlier childhood (ages 0-5 year average, age 78 months) blood Pb levels in children ages 15-17 years. The means for these blood Pb metrics ranged from 4.8 to 12 µg/dL. These associations were found with adjustment for several potential confounding factors, including SES, parental caregiving quality, and child health and without indication of substantial selection bias. Evidence from cross-sectional studies was less consistent. The biological plausibility for associations observed in children is provided by a study that found poorer balance in male mice with relevant gestational to early postnatal (PND10) Pb exposures. The evidence in children, particularly from the few prospective studies, and the coherence with limited available findings in mice is sufficient to conclude that a causal relationship is likely to exist between Pb exposure and decrements in motor function in children.

4.3.15.8 Evidence for Cognitive Function Decrements in Adults

A causal relationship is likely to exist between Pb exposure and cognitive function decrements in adults based on prospective and cross-sectional epidemiologic studies that indicate associations with bone Pb level and coherence with evidence in animals (Table 4-17). Key evidence for bone Pb levels was provided by recent prospective analyses of the BMS and NAS, with support provided by the cross-sectional Nurses' Health Study. The multiple risk factors and health outcomes examined in these studies reduces the likelihood of biased participation and/or follow-up by adults specifically with higher Pb exposure and lower cognitive function. While the NAS and Nurses' Health Study examined primarily white men and white women, respectively, the BMS examined a

more ethnically diverse population of men and women, increasing the generalizability of findings. There was variability in associations across the various domains of cognitive function tested within studies; however, bone Pb levels were associated with decrements in most of the neuropsychological tests performed. In many studies, bone Pb levels were associated with poorer executive function, visuospatial skills, learning, and memory.

Recent evidence from prospective analyses of the NAS and BMS cohorts expanded upon previous cross-sectional evidence by improving characterization of the temporal sequence between Pb exposure and cognitive function declines in adults (n = 358-965, mean ages 59-69 years) by demonstrating that higher tibia (means: 19, 20 μg/g) or patella (mean: 25 μg/g) bone Pb levels measured at baseline were associated with subsequent declines in cognitive function over 2- to 4-year periods (Bandeen-Roche et al., 2009; Weisskopf et al., 2007b). The specific potential confounding factors considered differed between studies; both studies adjusted for age and education. Additional adjustment was made for household wealth in the BMS and current alcohol use and current smoking in the NAS.

Evidence from most cross-sectional analyses supported associations between higher bone Pb level and decrements in cognitive function in adults. A strength of cross-sectional studies overall was the adjustment for many of the potential confounding factors described above and also dietary factors, physical activity, medication use, and comorbid conditions (Rajan et al., 2008; Weuve et al., 2006). Cross-sectional studies generally demonstrated larger decrements in cognitive function in adults per unit increase in tibia or patella Pb levels than concurrent blood Pb levels. Results from the NAS and Nurses' Health Study did not clearly indicate a difference in association with cognitive performance between tibia and patella Pb levels (Weuve et al., 2009; Weisskopf et al., 2007b). In NHANES analyses, higher concurrent blood Pb levels were associated with lower cognitive function in particular age and genetic variant subgroups but not consistently across the various cognitive tests conducted (Krieg et al., 2010; Krieg and Butler, 2009; Krieg et al., 2009). NHANES did not have bone Pb measures for comparison.

Because bone Pb is a major contributor to blood Pb levels, blood Pb level also can reflect longer term exposures, including higher past exposures, especially in adults without occupational exposures (Sections 3.3 and 3.7.3). Thus, in the NHANES results, it is difficult to characterize the relative contributions of recent and past Pb exposures to the associations observed between concurrent blood Pb level and cognitive function. The discrepant findings for blood and bone Pb levels in other studies indicate that cumulative Pb exposure that likely included higher past exposures may be a better predictor of cognitive function in adults than is concurrent blood Pb level. Additional support for the

effects of cumulative or past Pb exposure is provided by analyses of a few child cohorts as adults, which indicate that childhood tooth (from 1st/2nd grade) and blood (average to age 10 years) Pb levels are associated with decrements in cognitive function in adults ages 19-30 years (Mazumdar et al., 2011; Bellinger et al., 1994a). An uncertainty in the evidence for bone Pb levels is potential residual confounding by age. Increasing age is highly correlated with increasing bone Pb level (Section 3.3.5.2), and distinguishing Pb-related declines in cognitive function from age-related declines with model adjustment is difficult. One explanation for the more variable findings in adults than in children may be that cognitive reserve may compensate for the effects of Pb exposure on learning new information. Among current smelter workers, blood Pb-associated decrements in cognitive function were larger among workers with lower cognitive reserve (Bleecker et al., 2007a). Compensatory mechanisms may be overwhelmed with age and with higher long-term or cumulative Pb exposure represented by higher bone Pb levels.

Higher blood and bone Pb levels were associated with cognitive function decrements in adults with current or former occupational Pb exposures (Table 4-17). Some studies examined current workers with a mean concurrent blood Pb level of 31 μg/dL (Dorsey et al., 2006; Stewart et al., 2002). Among adults with current occupational Pb exposures, both concurrent and cumulative exposures may affect cognitive function. Several of these studies considered potential confounding by a similar set of factors as did studies of adults without occupational Pb exposures but did not examine other occupational exposures such as manganese. In the prospective study of adults with former occupational Pb exposure, peak tibia Pb levels were associated more strongly with cognitive performance than were concurrent blood Pb levels (Khalil et al., 2009a). Thus, in the absence of higher current Pb exposures, cumulative Pb exposures may have a greater effect on cognitive function in adults.

Additional support for a relationship between Pb exposure and cognitive function decrements in adults is provided by the coherence with evidence in adult animals that lifetime Pb exposure starting from gestation, birth, or after weaning induces learning impairments (Table 4-17 and Section 4.3.2.3). Biological plausibility also is provided by evidence describing the effects of Pb on modes of action underlying cognitive function. Cognitive function is mediated by actions of the neurotransmitters dopamine and glutamate in the hippocampus, prefrontal cortex, and nucleus accumbens. Experimental studies have shown that Pb induces changes in neurotransmitter release in these regions. Studies also have shown Pb-induced decreases in the magnitude of LTP, which is a major cellular mechanism underlying learning and memory.

In conclusion, in adults without occupational exposure, recent prospective studies in the NAS and BMS cohorts indicate associations of higher baseline tibia (means 19, 20 μg/g) or patella (mean 25 μg/g) Pb levels with declines in cognitive function in adults (>age 50 years) over 2- to 4-year periods. While the specific covariates differed between studies, these bone Pb-associated cognitive function decrements were found with adjustment for potential confounding factors such as age, education, SES, current alcohol use, and current smoking. Supporting evidence is provided by cross-sectional analyses of the NAS, BMS, and the Nurses' Health Study, which found stronger associations with bone Pb level than concurrent blood Pb level. Cross-sectional studies also considered more potential confounding factors, including dietary factors, physical activity, medication use, and comorbid conditions. The multiple exposures and health outcomes examined in many studies reduces the likelihood of biased participation by adults specifically with higher Pb exposure and lower cognitive function. The collective evidence indicates associations in cohorts of white men and women and a cohort of more ethnically diverse men and women. The specific timing, frequency, duration, and magnitude of Pb exposures contributing to the associations observed with bone Pb levels are uncertain. Also uncertain is the potential for residual confounding by age. The effects of recent Pb exposures on cognitive function decrements were indicated in Pb-exposed workers by associations found with blood Pb levels, although these studies did not consider potential confounding by other workplace exposures. The biological plausibility for the effects of Pb exposure on cognitive function decrements in adults is provided by findings that lifetime Pb exposures from gestation, birth, or after weaning induce learning impairments in adult animals and by evidence for the effects of Pb on altering neurotransmitter function in the hippocampus, prefrontal cortex, and nucleus accumbens. The associations between bone Pb level and cognitive function decrements consistently found in the few prospective and cross-sectional studies of adults without occupational Pb exposure, the coherence with animal findings, and toxicological evidence supporting modes of action, but uncertainties related to potential residual confounding by age in epidemiologic studies, are sufficient to conclude that a causal relationship is likely to exist between long-term cumulative Pb exposure and cognitive function decrements in adults.

4.3.15.9 Evidence for Psychopathological Effects in Adults

Evidence indicates that a causal relationship is likely to exist between Pb exposure and psychopathological effects in adults, based on the associations found between concurrent blood or bone Pb level and self-reported symptoms of depression, anxiety, and panic disorder in large cross-sectional studies of adults (i.e., NHANES, NAS) and some supporting toxicological evidence. Higher cord blood δ-ALA level was associated with

schizophrenia in adults in California (Opler et al., 2008; 2004), but because of the lack of assessment of blood or bone Pb levels, results were not a major consideration in conclusions. In both NHANES and NAS, the high participation rates and examination of multiple exposures and outcomes reduces the likelihood that findings are influenced by biased participation or reporting of symptoms by subjects specifically with higher Pb exposures. Symptoms of depression and anxiety were assessed with widely-used structured questionnaires such as the Profile of Mood States, but there is uncertainty regarding the effects of Pb exposure on clinically-diagnosed disorders.

Epidemiologic evidence for associations between blood or bone Pb level and psychopathological effects is strengthened by the adjustment for several potential confounding factors including age, education, smoking, alcohol use, employment status, and SES. Among adults ages 20-39 years participating in NHANES, 369 adults with concurrent blood Pb level ≥ 2.1 µg/dL had the highest OR for self-reported major depressive disorder (OR: 2.3 [95% CI: 1.1, 4.8]) and panic disorder (OR: 4.9 [95% CI: 1.3, 18]) compared with the 449 adults with blood Pb levels 0.3-0.7 µg/dL (Bouchard et al., 2009). Among NAS men ages 48-94 years, higher concurrent blood, patella, and tibia Pb levels were associated with self-reported anxiety (nervousness, tension), depression, and phobic anxiety (a subcategory of anxiety but defined specifically as persistent fear, irrational and disproportionate to a stimulus) (Rajan et al., 2007; Rhodes et al., 2003). A 14 µg/g increase in tibia Pb level was associated with a self-reported anxiety score one standard deviation above the mean with an OR of 1.2 (95% CI: 0.98, 1.4) (Rajan et al., 2007). Because of the cross-sectional design of studies, the temporal sequence between Pb exposure and psychopathological symptoms in adults is uncertain. This uncertainty is somewhat reduced with results for tibia Pb, since it is an indicator of cumulative Pb exposure. For results with blood and bone Pb level, there is uncertainty regarding the specific level, timing, frequency, and duration of Pb exposure associated with psychopathological effects.

The epidemiologic evidence for Pb-associated psychopathological effects is supported by the coherence with findings in rodents that dietary lactational Pb exposure with or without additional post-lactational exposure resulted in depression-like behavior and emotionality in rodents, with some evidence at blood Pb levels relevant to this ISA (13, 17 µg/dL) (Beaudin et al., 2007; Dyatlov and Lawrence, 2002). Other studies found Pb-induced increases in depression-like behavior in animals with higher than relevant blood Pb levels (Section 4.3.4.2). Further support for Pb-associated increases in psychopathological effects in adults is provided by evidence that describes modes of action, including Pb-induced changes in the HPA axis (Section 4.3.2.3) and dopaminergic and GABAergic systems (Sections 4.2.2.2, 4.3.10.4, and 4.3.10.8), which are found to affect mood and emotional state.

In conclusion, cross-sectional studies in a few populations demonstrate associations of higher concurrent blood, patella, or tibia Pb levels with self-reported symptoms of depression and anxiety in adults. The examination of multiple exposures and outcomes in the available studies does not provide strong indication of biased reporting of psychopathological effects by adults specifically with higher Pb exposures. In adults, Pb-associated increases in symptoms of depression and anxiety were found with adjustment for age, SES, education, and in the NAS, daily alcohol intake and pack-years smoking. The biological plausibility for epidemiologic evidence is provided by observations of depression-like behavior or emotionality in animals with dietary lactational Pb exposure, with some evidence at blood Pb levels relevant to this ISA and by toxicological evidence supporting modes of action, including Pb-induced changes in the HPA axis and dopaminergic and GABAergic systems. The associations of blood and bone Pb level with symptoms of psychopathological effects found in the few studies of adults without occupational Pb exposure as well as the biological plausibility provided by the coherence with findings from a few rodent studies and evidence for underlying modes of action, but uncertainties related to residual confounding of bone Pb results by age in epidemiologic studies are sufficient to conclude that a causal relationship is likely to exist between Pb exposure and psychopathological effects in adults.

4.3.15.10 Evidence for Auditory Function Decrements in Adults

The evidence is suggestive of a causal relationship between Pb exposure and auditory function decrements in adults based primarily on findings from a few epidemiologic studies. Key evidence in humans is provided by the recent analysis of NAS males in which a 15 µg/g higher tibia Pb level at mean age 64.9 years was associated with a 0.05 dB/year (95% CI: 0.017, 0.083) increase in hearing threshold for a pure tone average frequency (Park et al., 2010). Results were adjusted for baseline age, race, education, occupational noise, BMI, pack-years smoking, noise notch, diabetes, and hypertension. Although the generalizability of results in this primarily white population of men is limited, high follow-up participation and the examination of multiple exposures and outcomes in this cohort reduces the likelihood that findings are biased by selective participation of men specifically with higher Pb exposures. Bone Pb levels were measured up to 20 years after the initial hearing measurement; however, tibia Pb level is considered an indicator of cumulative Pb exposure since the half-life of Pb in bone is on the order of decades (Section 3.3). Bone Pb levels increase with age, and although age was included as a model covariate, residual confounding by age is possible. Supporting evidence was provided by a recent case-control study of adults attending a hospital for occupational health exams. Despite limitations of a nonrandom population and uncertain

comparability of controls, the examination of multiple metals reduces the likelihood of biased participation by higher Pb exposure. Higher concurrent blood Pb level was associated with hearing loss with adjustment for several factors including blood levels of Mn, As, and Se (Chuang et al., 2007).

Pb-associated increases in hearing thresholds or latencies in brainstem auditory evoked potentials were found in adult monkeys ages 8-13 years with lifetime Pb exposure in drinking water from gestation or birth (Rice, 1997; Lilienthal and Winneke, 1996). A recent study found small increases in hearing thresholds in monkeys at age 13 years with Pb exposure only during gestation or lactation (to age 5.5 months) (Laughlin et al., 2009). However, these effects were found with higher blood Pb levels (i.e., at peak or testing 33-150 µg/dL) than those relevant to this ISA; thus, the biological plausibility for epidemiologic observations is unclear.

In conclusion, the evidence provided by the analysis of NAS men for associations of higher tibia Pb level with a higher rate of elevations in hearing threshold over 20 years but uncertainties related to effects on auditory function in adult animals with relevant Pb exposures, is suggestive of a causal relationship between Pb exposure and auditory function decrements in adults.

4.3.15.11 Evidence for Visual Function Decrements in Adults

The effects of relevant Pb exposures on visual function related to CNS changes, specifically in adults have been examined in some toxicological studies, but epidemiologic evidence is sparse. Studies in adult animals show differential effects on ERGs, depending on the timing and concentration of Pb exposure. Although studies have linked Pb-induced ERG changes to changes in rod cell proliferation, retinal cell apoptosis, and dopamine, the implications of findings are unclear because the functional relevance of the observed ERG changes is unclear. Decreased visual acuity was found in adult monkeys with lifetime (to age 7-9 years) Pb exposure but at higher concentrations than those relevant to this ISA, 50-115 µg/dL (Rice, 1998). A case-control study found higher Pb concentrations in retinal tissue of macular degeneration adult cases (Table 4-17) but was limited by the inability to establish directionality of effects, the uncertain representativeness of the control population, and the lack of rigorous statistical analysis or examination of potential confounding factors. Because the available epidemiologic and toxicological evidence is of insufficient quantity, quality, and consistency, the evidence is inadequate to determine that a causal relationship exists between Pb exposure and visual function decrements in adults.

4.3.15.12 Evidence for Neurodegenerative Diseases in Adults

Epidemiologic and toxicological studies combined produced inconclusive evidence for Alzheimer's disease and ALS but found associations between indicators of Pb exposure and neurodegenerative diseases such as Parkinson's disease and essential tremor. However, study limitations as described below limit the implications of findings.

The few case-control studies of essential tremor found higher concurrent blood Pb levels in cases than controls (Section 4.3.9.4). A common limitation of these studies was the potential for reverse causation. Reduced physical activity among cases could result in greater bone turnover and greater release of Pb from bones into blood in cases than controls. Some case-control studies found adults with Parkinson's disease to have higher bone Pb levels, which are not likely to increase with decreases in physical activity (Weisskopf et al., 2010; Coon et al., 2006). While some of these studies of Parkinson's disease and essential tremor considered potential confounding by factors such as age, sex, race, education, and alcohol consumption, they did not consider Mn co-exposure. Epidemiologic findings for Parkinson's disease are supported by limited available mode of action evidence for Pb-induced decreased dopaminergic cell activity in the substantia nigra, which contributes to the primary symptoms of Parkinson's disease.

The few case-control studies of Alzheimer's disease did not find higher prevalence of occupational Pb exposure or higher brain Pb levels in cases but did not measure Pb in blood or bone (Section 4.3.9.1). Toxicological studies indicated that gestational-lactational or infancy Pb exposure of monkeys and rats (but not adult-only exposure in rats) induced pathologies that underlie Alzheimer's disease, including the formation of amyloid plaques and neurofibrillary tangles in the brains of aged animals (Section 4.3.9.1). While these results suggest the need to consider early-life Pb exposure in epidemiologic studies, some indicate that effects may be attributable to the high Pb exposure concentrations tested, i.e., producing blood Pb levels >40 µg/dL in rats (Li et al., 2010b; Basha et al., 2005). Studies of ALS have not consistently found higher Pb biomarker levels or history of occupational Pb exposure among ALS cases and controls (Section 4.3.9.2), and a recent study found that higher tibia and patella Pb levels were associated with longer survival time among ALS cases (Kamel et al., 2008).

In conclusion, while evidence is inconclusive for ALS and Alzheimer's disease, a few case-control studies each found higher blood Pb levels in adults with essential tremor and higher bone Pb levels in adults with Parkinson's disease. Because of the inconclusive evidence for some diseases and limitations such as reverse causation for essential tremor and the lack of consideration for potential confounding by Mn exposure for both essential tremor and Parkinson's disease, the evidence is inadequate to determine that there is a causal relationship between Pb exposure and neurodegenerative diseases.

Table 4-17 Summary of Evidence Supporting Nervous System Causal Determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Biomarker Levels Associated with Effects[c]
Cognitive Function Decrements in Children - Causal			
Consistent associations from multiple, high quality epidemiologic studies with relevant blood Pb levels	Evidence from prospective studies for decrements in FSIQ in association with prenatal, earlier childhood, peak, concurrent, lifetime average blood Pb levels and tooth Pb levels in children ages 4-17 yr in multiple U.S. locations, Mexico, Europe, Australia	Canfield et al. (2003a), Bellinger et al. (1992), Jusko et al. (2008), Dietrich et al. (1993b), Schnaas et al. (2006), Wasserman et al. (1997), Tong et al. (1996), Lanphear et al. (2005) Plus Table 4-3, Section 4.3.2.1	Blood Pb (various time periods & lifestages): Means 3-16 µg/dL With consideration of peak or early childhood blood Pb levels: Means 3-8 µg/dL for concurrent (age 4, 5 yr), age 2 yr
	Evidence from prospective studies for lower scores on tests of executive function and academic performance in association with earlier childhood or lifetime average blood Pb levels or tooth Pb levels in children ages 5-20 yr in multiple U.S. locations, U.K, New Zealand. Associations less consistent for learning and memory.	Canfield et al. (2004), Stiles and Bellinger (1993), Miranda et al. (2009; 2007a), Fergusson et al. (1997, 1993), Leviton et al. (1993), Chandramouli et al. (2009) Sections 4.3.2.3, 4.3.2.4, 4.3.2.5	Blood Pb (various time periods & lifestages): Means 4.8-7.2 µg/dL, Groups with early childhood blood Pb 2-16 µg/dL and 5-10 µg/dL Tooth Pb (ages 6-8 yr): means 3.3, 6.2 µg/g
	Supporting evidence from cross-sectional studies of children ages 3-16 yr, but most did not consider potential confounding by parental caregiving quality. Includes large NHANES III analysis.	Surkan et al. (2007), Kim et al. (2009b), Roy et al. (2011), Lanphear et al. (2000), Froehlich et al. (2007), Chiodo et al. (2007; 2004)	Concurrent (ages 3-16 yr) blood Pb : Means 1.7-12 µg/dL, Group (ages 6-10 yr) with blood Pb 5-10 µg/dL
	Outcomes assessed using widely-used, structured questionnaires.		
	Several studies indicate supralinear C-R relationship, with larger decrements in cognitive function per unit increase in blood Pb at lower blood Pb levels in children ages 5-10 yr	Canfield et al. (2003a), Bellinger et al. (1992), Jusko et al. (2008), Kordas et al. (2006), Lanphear et al. (2005) Plus Table 4-16	Groups with peak blood Pb <10 µg/dL: concurrent mean 3.3 µg/dL, age 2 year mean 3.8 µg/dL

Table 4-17 (Continued): Summary of Evidence Supporting Nervous System Causal Determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Biomarker Levels Associated with Effects[c]
Additional epidemiologic evidence to help rule out chance, bias, and confounding with reasonable confidence	Several epidemiologic studies found associations with adjustment for SES, maternal IQ and education, HOME score. Several adjust for birth weight, smoking. A few, nutritional factors. Epidemiologic studies had population-based recruitment, most with moderate to high follow-up participation not conditional on blood or tooth Pb level or cognitive function.	Table 4-3, Table 4-5; Table 4-8, Table 4-9, Sections 4.3.2.1, 4.3.2.3 4.3.2.4, and 4.3.2.5	
	Pooled and meta-analyses demonstrate the consistency of association	Lanphear et al. (2005), Pocock et al. (1994), Schwartz (1994)	
Consistent evidence in animals with relevant exposures to help rule out chance, bias, and confounding with reasonable confidence	Impaired learning and associative ability in juvenile and adult animals as indicated by performance in tasks of visual discrimination, water maze, y maze, and operant conditioning with schedules of reinforcement with relevant dietary Pb exposure.	Stangle et al. (2007), Niu et al. (2009), Cory-Slechta et al. (2010), Altmann et al. (1993), Section 4.3.2.3	Blood Pb (after prenatal/ lactation, lactation only, prenatal/lifetime Pb exposure): 10-25 µg/dL
	Impaired learning, memory, executive function in adult monkeys as indicated by poorer performance on delayed spatial alternation and spatial discrimination reversal learning tasks with dietary Pb exposures.	Gilbert and Rice (1987), Rice and Karpinski (1988), Sections 4.3.2.3 and 4.3.2.4	Blood Pb (after lifetime Pb exposure from birth): 15, 25 µg/dL

Table 4-17 (Continued): Summary of Evidence Supporting Nervous System Causal Determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Biomarker Levels Associated with Effects[c]
Evidence describes mode of action:	Decreased neurogenesis in hippocampus DG (involved in LTP and learning). Decreased NMDAR (involved in integration of new neurons into existing neuronal pathways). Decreased neurite outgrowth.	Sections 4.3.10.9 and 4.3.10.10	
Impaired neuron development	Found in animals with dietary gestational-lactational, lactational, post-lactational (3-8 weeks), lifetime from gestation Pb exposures.		
Synaptic changes	Decreased synaptic development. Changes in synaptic protein composition. Decreased ATP and AchE, which both mediate neurotransmission.	Section 4.3.10.4	
	Found in animals with dietary gestational with or without additional lactational Pb exposures.		
LTP	Decreased magnitude, increased threshold of LTP with gestational-lactational or lifetime Pb exposure.	Sections 4.3.12, 4.3.10.7, 4.3.10.8	
Neurotransmitter changes	Changes in dopamine metabolism. Increased sensitivity of dopamine receptor. Increased catecholamine transmission in cerebral cortex, cerebellum, hippocampus. Decreased glutamate and expression of glutamate receptor, NMDAR.	Section 4.3.10.8	
	Found in animals with dietary gestational-lactational, lactational, or post-lactational Pb exposure.		

Table 4-17 (Continued): Summary of Evidence Supporting Nervous System Causal Determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Biomarker Levels Associated with Effects[c]
Externalizing Behaviors: Attention, Impulsivity, and Hyperactivity in Children – Causal			
Consistent associations from multiple, high quality epidemiologic studies with relevant blood Pb levels	Evidence from prospective studies for attention decrements and hyperactivity in association with prenatal (maternal or cord), early childhood, and lifetime avg blood Pb and tooth Pb levels in children ages 7-17 yr and young adults 19-20 yr in U.S., U.K., Australia, New Zealand.	Burns et al. (1999), Ris et al. (2004), Fergusson et al. (1993), Bellinger et al. (1994a), Chandramouli et al. (2009), Leviton et al. (1993) Section 4.3.3.1	Blood Pb: Means 6.8, 8.3 µg/dL (prenatal maternal or cord), 8.3 µg/dL age 6 yr, 13.4 age 3-60 month, 14 µg/dL (lifetime avg to age 11-13 yr) Group with age 30 mo >10 µg/dL Tooth Pb (ages 6-8 yr): Means: 3.3, 6.2 µg/g
	Association not found with ratings of attention problems in children ages 4-5 yr in whom ratings may be measured less reliably	Wasserman et al. (2001) Section 4.3.3.1	Blood Pb: Mean 7.2 µg/dL for lifetime (to age 4-5 yr) avg
	Associations found with neuropsychological tests (CPT) and teacher and parent ratings using widely-used, structured questionnaires.		
Consistent associations from multiple, high quality epidemiologic studies with relevant blood Pb levels (Continued)	Supporting evidence from higher quality cross-sectional studies (consideration for several potential confounding facors) for associations of concurrent blood Pb level with attention decrements, impulsivity, and hyperactivity in children ages 5-7.5 yr. Some populations had high prenatal drug or alcohol exposure.	Chiodo et al. (2007; 2004) Plusquellec et al. (2010) Section 4.3.3.1	Concurrent (ages 5-7.5 yr) blood Pb: Means 5.0-5.4 µg/dL
	No association in Rochester cohort, age 4.5 yr.	Canfield et al. (2003b)	Concurrent (age 4.5 yr) blood Pb: Mean 6.5 µg/dL
	Associations found in most other cross-sectional studies with less extensive consideration for potential confounding	Section 4.3.3.1, Table 4-11	Concurrent (ages 3-17 yr) blood Pb: Means 1-12 µg/dL
Additional epidemiologic evidence to help rule out chance, bias, and confounding with reasonable confidence	Most prospective and some cross-sectional studies found associations with adjustment for SES, maternal education, and parental caregiving quality. Some also considered parental IQ, smoking, birth outcomes. A few considered substance abuse, nutritional factors. Studies had population-based recruitment with moderate to high follow-up participation not conditional on blood or tooth Pb level.	Section 4.3.3.1, Table 4-11	

Table 4-17 (Continued): Summary of Evidence Supporting Nervous System Causal Determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Biomarker Levels Associated with Effects[c]
Consistent evidence in animals with relevant exposures to help rule out chance, bias, and confounding with reasonable confidence	Impulsivity indicated by premature responses, increased response perseveration, decreased pause time between events on tests with FI, FR/waiting for reward, or Differential reinforcement of low respose rate in rodents and monkeys with relevant dietary postnatal Pb exposures.	Rossi-George et al. (2011), Brockel and Cory-Slechta (1999b, 1998), Rice and Gilbert (1985), Gilbert and Rice (1987) Section 4.3.3.1	Blood Pb: 15, 25 µg/dL in monkeys with lifetime exposure, 11-16 µg/dL in rats with lifetime exposure from gestation; 11-29 µg/dL in rats with 3, 7 mo post-weaning exposure
	Inconsistent evidence for sustained attention in rats and monkeys with relevant postnatal dietary Pb exposures. Increased activity in male mice found only with Pb and amphetamine co-exposure.	Sustained attention: Gilbert and Rice (1987), Brockel and Cory-Slechta (1999a) Hyperactivity: Leasure et al. (2008) Section 4.3.3.1	Blood Pb: 15, 25 µg/dL in monkeys after lifetime (after birth) exposure; 16, 28 µg/dL in rats after 34-day post-weaning exposure
Evidence describes mode of action	Same as above for cognitive function in children		

Table 4-17 (Continued): Summary of Evidence Supporting Nervous System Causal Determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Biomarker Levels Associated with Effects[c]
Externalizing Behaviors: Conduct Disorders in Children and Young Adults (e.g., delinquent, aggressive, antisocial behavior, and criminal offenses) – **Likely Causal**			
Consistent results from a few high-quality epidemiologic studies with relevant blood or tooth Pb levels	Evidence from prospective studies for higher parent and teacher ratings of aggressive, antisocial, delinquent behavior in children ages 8-17 yr in U.S., U.K., Australia in association with earlier childhood or lifetime average blood Pb levels.	Dietrich et al. (2001), Burns et al. (1999), Chandramouli et al. (2009), Section 4.3.3.2	Blood Pb: lifetime (to age 11-13 yr) avg mean 14 µg/dL, age 6 year mean 12 µg/dL, group with age 30 month blood Pb >10 µg/dL
	Evidence from prospective studies for criminal offenses in young adults ages 19-24 yr in Cincinnati and New Zealand in association with earlier childhood blood or tooth Pb levels.	Wright et al. (2008), Fergusson et al. (2008), Section 4.3.3.2	Age 6 yr blood Pb mean 6.8 µg/dL Tooth Pb (age 6-8 yr) mean: 6.2 µg/g
	Association found in Yugoslavia cohort at ages 4-5 years, when behavior ratings may be less reliable.	Wasserman et al. (2001)	Mean lifetime (to ages 4-5 yr) avg: 7.2
	Studies had moderate to high follow-up participation, not conditional on blood Pb level. Most studies considered potential confounding by SES, parental IQ and education, other SES factors, parental caregiving quality, family functioning, parental smoking or substance abuse.	Table 4-12	
	Supporting evidence from cross-sectional studies of children ages 7-15 years, including NHANES analysis and study of children in Detroit, MI that had extensive consideration for potential confounding.	Braun et al. (2008) Chiodo et al. (2007) Section 4.3.3.2	Groups ages 8-17 years with concurrent blood Pb 0.8-10 µg/dL Concurrent (age 7 year) blood Pb mean: 5.0 µg/dL
	Consistency supported by meta-analysis indicating similar effect estimates by study design, potential confounding factors considered Teacher and parental ratings derived from widely-used, structured questionnaires.	Marcus et al. (2010) Section 4.3.3.2	Blood Pb range of study means (concurrent, lifetime avg): 1.0-26 µg/dL

Table 4-17 (Continued): Summary of Evidence Supporting Nervous System Causal Determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Biomarker Levels Associated with Effects[c]
But, uncertainty because of inconsistent animal evidence with relevant exposures and small number of epidemiologic studies	Aggression observed in adult hamsters with gestational-lifetime dietary Pb exposure. Other evidence in adult animals with similar duration exposure inconsistent. Aggression generally not found in juvenile animals with lactational Pb exposure. No specific mode of action examined with Pb exposure.	Delville (1999) Section 4.3.3.2	Blood Pb after gestational-lifetime exposure: 10-15 µg/dL
Internalizing Behaviors in Children (e.g., withdrawn behavior, symptoms of depression, anxiety, fearfulness) **– Likely Causal**			
Consistent results from a few high-quality epidemiologic studies with relevant exposures	Evidence from prospective studies for higher ratings of internalizing behaviors in children ages 8-13 yr in Boston and Port Pirie cohorts in association with tooth or lifetime average blood Pb levels.	Burns et al. (1999), Bellinger et al. (1994b) Section 4.3.4.1	Blood Pb lifetime (to age 11-13 yr) average mean: ~14 µg/dL Tooth Pb (age 6 yr) mean: 3.4 µg/g
	Associations also found in children age 4-5 yr in Yugoslavia in association with lifetime average blood Pb level; stronger association found for delinquent behavior. Studies had population-based recruitment with moderate follow-up participation. Participation not conditional on tooth/blood Pb levels and behavior. Associations found with teacher and parent ratings on widely used, structured questionnaires.	Wasserman et al. (2001)	Blood Pb lifetime (to age 4-5 yr) average mean: 7.2 µg/dL
	Inconsistent associations in cross-sectional studies that had extensive consideration for potential confounding and examined relatively low concurrent blood Pb levels.	Chiodo et al. (2004) Plusquellec et al. (2010) Section 4.3.4.1	Concurrent blood Pb means (ages 5, 7 yr): 5, 5.4 µg/dL
And, supporting animal evidence with relevant exposures from a few studies	Dietary lactational Pb exposure increased emotionality, depression-like behavior in juvenile rodents	Beaudin et al. (2007) Dyatlov and Lawrence (2002) Section 4.3.4.2	Blood Pb at PND52 after PND1-PND30 exposure: 13 µg/dL Blood Pb at PND22 after PND1-PND22 exposure: 17 µg/dL
Some evidence describing mode of action	Pb-induced changes in HPA axis and dopaminergic and GABAergic systems	Sections 4.3.2.3, 4.2.2.2, 4.3.10.4, 4.3.10.8	

Table 4-17 (Continued): Summary of Evidence Supporting Nervous System Causal Determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Biomarker Levels Associated with Effects[c]
But, uncertainty regarding potential confounding	Epidemiologic associations found with adjustment for SES, birth outcomes, parental education. Few studies adjusted for or considered HOME score. Uncertainty regarding potential confounding by parental caregiving quality.	Burns et al. (1999), Bellinger et al. (1994b), Wasserman et al. (2001) Chiodo et al. (2004) Plusquellec et al. (2010) Section 4.3.4.1	
Auditory Function Decrements in Children – Likely Causal			
Consistent findings from a few high-quality epidemiologic studies with relevant blood Pb levels	Prospective study found associations of prenatal (maternal), neonatal, yearly age 1 to 5 yr, lifetime avg blood Pb levels with poorer auditory processing in children at age 5 yr in Cincinnati. Information was not provided on participation rates. Results were adjusted for SES, HOME score, birth outcomes, obstetrical complications, maternal smoking. Several other factors considered.	Dietrich et al. (1992) Section 4.3.6.1	Blood Pb means: neonatal (10 day) 4.8 µg/dL, yearly age 1 to 5 year 10.6-17.2 µg/dL, lifetime (to age 5 yr) avg NR
	Supporting evidence from cross-sectional studies for increased hearing thresholds in children ages 4-19 yr participating in NHANES and HHANES in association with higher concurrent blood Pb levels. Examination of multiple exposures and outcomes in NHANES and HHANES reduces likelihood of selection bias specifically by blood Pb levels. Studies considered potential confounding by age, sex, race, income, parental education, nutritional factors.	Schwartz and Otto (1991, 1987) Section 4.3.6.1	Blood Pb median: HHANES: 8 µg/dL NHANES: NR
But, uncertainty because of lack of animal evidence in juveniles and at relevant exposures	Increased hearing thresholds, decreased auditory evoked potentials in adult monkeys (ages 8-13 years) with lifetime dietary Pb exposure.	Rice (1997) Lilienthal and Winneke (1996) Section 4.3.6.1	Blood Pb after lifetime exposure: 33-150 µg/dL
	Changes in neurotransmitters in regions of brain involved in auditory integration in mice with gestational-lactational Pb exposure	Fortune et al. (2009) Section 4.3.10.8	Blood Pb after gestational-lactational exposure: 8.2, 42 µg/dL

Table 4-17 (Continued): Summary of Evidence Supporting Nervous System Causal Determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Biomarker Levels Associated with Effects[c]
Visual Function Decrements in Children - Inadequate			
The available evidence is of insufficient quantity, quality, or consistency	High maternal 1st trimester blood Pb associated with supernormal ERG in a study of children. No potential confounding considered. Uncertain functional relevance of ERG findings.	Rothenberg et al. (2002b) Section 4.3.6.2	Group with prenatal maternal blood Pb 10.5-32.5 µg/dL
	Higher than relevant Pb exposures did not affect visual acuity in infant monkeys but decreased visual acuity in adult monkeys.	Laughlin et al. (2008) Section 4.3.6.2	Blood Pb after postnatal day 8-week 26 exposure: 35-40 µg/dL
	Examination of retinal ERGs limited to adult rats (see below for Visual Function Decrements in Adults)	Section 4.3.6.2	
Motor Function Decrements in Children – Likely Causal			
Consistent findings from a few high-quality epidemiologic studies with relevant blood Pb levels	Evidence from prospective studies for fine and gross motor function decrements in children ages 4.5-17 yr in Cincinnati, Yugoslavia in association with neonatal, earlier childhood, concurrent, lifetime avg blood Pb levels. High follow-up participation, no selective attrition in Cincinnati cohort, higher loss-to-follow-up in Yugoslavia cohort with lower maternal IQ, HOME. Both studies adjusted for maternal IQ, parental education, SES, HOME score Studies used various, widely-used tests to assess outcomes.	Ris et al. (2004), Dietrich et al. (1993a), Bhattacharya et al. (2006; 1995), Wasserman et al. (2000b) Section 4.3.7	Blood Pb means Cincinnati: neonatal (10 day) 4.8 µg/dL, concurrent (age 6 yr) 11.6 µg/dL, lifetime (to age 15-17 yr) avg 12.3 µg/dL, age 0-5 yr avg 11.7 µg/dL Yugoslavia: NR
	Mixed evidence for lower (concurrent) blood Pb levels from cross-sectional studies that considered several potential confounding factors.	Section 4.3.7	
	No decrease in motor function found in children ages 6-10 yr in New England.	Surkan et al. (2007)	Concurrent blood Pb mean: 2.2 µg/dL
	Decrease found in Inuit children ages 4-6 years in Canada.	Despres et al. (2005),	Concurrent blood Pb mean: 4.1 µg/dL

Table 4-17 (Continued): Summary of Evidence Supporting Nervous System Causal Determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Biomarker Levels Associated with Effects[c]
But, uncertainty because of limited animal evidence at relevant exposures	Poorer balance (fell off rotarod more quickly) in adult mice with gestational-lactation dietary Pb exposure	Leasure et al. (2008) Section 4.3.7	Peak blood Pb after gestational -lactational exposure ~10 µg/dL
	Inconsistent results with higher than relevant postnatal exposures	Section 4.3.7	Blood Pb after 2-7 week postnatal Pb exposure >59 µg/dL
Cognitive Function Decrements in Adults – Likely Causal			
Consistent findings from high-quality epidemiologic studies with relevant bone Pb levels	Prospective analyses in NAS cohort of white men and BMS cohort of men and women of diverse ethnicities found cognitive function decrements over 2 to 4 years in association with bone Pb levels. Baseline participation rates differed but high follow-up participation, not conditional on bone Pb levels. Different potential confounding factors considered. Adjustment for age and education in both cohorts, SES in BMS, smoking and alcohol use in NAS.	Weisskopf et al. (2007b), Bandeen-Roche et al. (2009) Table 4-10 and Section 4.3.2.7	Baseline means: 19, 20 µg/g tibia 25 µg/g patella
	Supporting cross-sectional evidence from NAS, BMS, and also women in Nurses' Health Study with adjustment for additional potential confounding factors, including dietary factors, medications, physical activity, comorbid conditions.	Nurses Healthy Study: Weuve et al. (2009) Section 4.3.2.7 Table 4-10	Concurrent means: 11 µg/g tibia 13 µg/g patella
	Associations with concurrent blood Pb level found in men and women participating in NHANES in certain age and genetic variant groups but not consistently.	Krieg et al. (2009), Krieg et al. (2010), Krieg and Butler (2009) Section 4.3.2.7	Concurrent blood Pb means: 3-4 µg/dL
	Some studies found associations in former and current Pb-exposed workers with relevant blood Pb levels and bone Pb levels. Most studies adjusted for age and education. Some also adjusted for depression and/or alcohol use; none considered other occupational exposures such as Mn. Outcomes assessed using various but widely used, structured instruments.	Dorsey et al. (2006), Schwartz et al. (2002) Stewart et al. (2002) Section 4.3.2.7	Concurrent blood Pb means: 31 µg/dL Peak tibia Pb mean: 26 µg/g.

Table 4-17 (Continued): Summary of Evidence Supporting Nervous System Causal Determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Biomarker Levels Associated with Effects[c]
Consistent evidence in animals with relevant exposures	Impaired learning, memory, and executive function in monkeys ages 7-10 years with lifetime dietary Pb exposures from birth. Impaired learning in animals with lifetime dietary Pb exposures starting in gestation.	Rice and Karpinski (1988), Gilbert and Rice (1987) Section 4.3.2.3 See above for cognitive function in children.	Blood Pb at age 200 days: 15, 25 µg/dL
Evidence clearly describes mode of action	Same as above for cognitive function in children		
But, important uncertainty remains	Few prospective studies available to address reverse causation Uncertainty regarding potential residual confounding of bone Pb results by age		
Psychopathological Effects in Adults (e.g., symptoms of depression, anxiety, panic disorder) **– Likely Causal**			
Consistent findings from a few high-quality epidemiologic studies with relevant blood and bone Pb levels	A few population-based cross-sectional studies indicate associations of higher concurrent blood, patella, or tibia Pb level with increased reporting of symptoms of depression, anxiety, panic disorder in adults. Studies had high participation rates and examined multiple exposures and outcomes and include NHANES analysis of 1,987 adults. Studies used widely used, structured instruments to assess outcomes but not diagnosed conditions.	Bouchard et al. (2009), Rhodes et al. (2003), Rajan et al. (2008) Section 4.3.5.1 Bouchard et al. (2009) Section 4.3.5.1	Concurrent blood Pb mean: 6 µg/dL Tibia Pb mean: 22 µg/g Patella Pb mean: 31, 32 µg/g Group with concurrent blood Pb ≥ 2.11 µg/dL
And, some supporting animal evidence with relevant exposures	Same as above for internalizing behaviors in children.		
But, uncertainty regarding potential confounding	Associations found with adjustment for education, age, and SES. Mn not considered. Also alcohol use and pack-years smoking in NAS. However, uncertainty regarding generalizability because of limited number of studies and potential for residual confounding of bone Pb results by age.	Section 4.3.5.1 Rhodes et al. (2003), Rajan et al. (2007)	

Table 4-17 (Continued): Summary of Evidence Supporting Nervous System Causal Determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Biomarker Levels Associated with Effects[c]
Auditory Function Decrements in Adults – Suggestive			
Limited epidemiologic evidence with relevant bone or blood Pb levels, one high-quality study	Prospective study found association between tibia Pb level and higher rate of increase in hearing threshold over 23 yr in NAS. Population comprises only males, primarily white, but study examines multiple exposures and outcomes and has high follow-up participation. Results adjusted for age, race, BMI, education, diabetes, hypertension, smoking, occupational noise.	Park et al. (2010) Section 4.3.6.1	Tibia Pb mean: 22.5 µg/g, measured near end of follow-up
	Supporting evidence from case-control study finding higher blood Pb levels in workers from various occupations with hearing loss with adjustment for age, smoking, alcohol consumption, years of noise exposure, blood Mn, As, Se	Chuang et al. (2007) Section 4.3.6.1	Concurrent blood Pb geometric mean in cases: 10.7 µg/dL
Lack of animal evidence at relevant exposures	Increased hearing thresholds, decreased auditory evoked potentials in adult monkeys (ages 8-13 years) with gestational, lactational, lifetime dietary Pb exposure.	Rice (1997) Lilienthal and Winneke (1996) Laughlin et al. (2009) Section 4.3.6.1	Blood Pb after gestational, lactational, or lifetime exposure: 33-150 µg/dL
Visual Function Decrements in Adults - Inadequate			
The available evidence is of insufficient quantity, quality, or consistency	Epidemiologic evidence limited to case-control study of higher Pb in retinal tissue of macular degeneration adult cases. Potential confounding not considered.	Erie et al. (2009)	Median Pb in retina tissue Cases: 12.0 ng/g Controls: 8.0 ng/g
	Higher than relevant Pb exposures decreases visual acuity in adult monkeys but not in infant monkeys.	Rice et al. (1998)	Blood Pb: 50-115 µg/dL with lifetime exposure to age 7-9 yr
	Supernormal or subnormal ERGs in adult rats depending on timing and dose of Pb exposure that were linked to changes in rod cell proliferation, retinal cell apoptosis, dopamine. Uncertain functional relevance.	Fox et al. (2008; 1997), Section 4.3.6.2	Blood Pb: 12, 24 µg/dL with gestational-lactational exposure, 19 µg/dL with lactational exposure

Table 4-17 (Continued): Summary of Evidence Supporting Nervous System Causal Determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Biomarker Levels Associated with Effects[c]
Neurodegenerative Diseases (e.g., Alzheimer's disease, ALS, Parkinson's disease, Essential tremor) **– Inadequate**			
The available evidence is of insufficient quantity, quality, or consistency	The few available case-control studies found higher blood or bone Pb levels or whole-body lifetime burden (PBPK modeling) in cases with Parkinson's disease and essential tremor. Results for ALS are mixed, association found with increased survival time.	Parkinson's disease: Section 4.3.9.3 Weisskopf et al. (2010), Coon et al. (2006)	Parkinson's disease: group with tibia Pb levels >16 µg/g, whole-body lifetime burden ≥ 81 µg/g
	Studies subject to selection and recall bias and reverse causation (for blood Pb studies) that could produce artifactual associations.	Essential tremor: Section 4.3.9.4 Louis et al. (2005; 2003), Dogu et al. (2007)	Essential tremor: concurrent blood Pb means 2.7-3.5 µg/dL
	Some studies consider potential confounding by age, sex, smoking, alcohol use, education, and activity levels, but not Mn exposures.	ALS: Section 4.3.9.2 Kamel et al. (2008; 2005; 2002), Vinceti et al. (1997), Fang et al. (2010)	ALS: Groups with concurrent blood Pb 3-14 µg/dL, concurrent blood Pb means 2.4, 12.7 µg/dL
	Case-control studies did not show associations between occupational history of Pb exposure or brain Pb levels and Alzheimer's disease.	Graves et al. (1991), Hariguchi et al. (2001), Section 4.3.9.1	
Some evidence describes mode of action	Amyloid plaques found in brains of adult monkeys and rodents with infancy-only Pb exposures.	Section 4.3.9.1	
	Effects in monkeys at ages 20-23 yr when blood Pb levels had returned to baseline.	Wu et al. (2008a)	Blood Pb with exposure to age 400 days: peak 32-36 µg/dL
	In rodents, no effect with adult-only Pb exposure.	Basha et al. (2005)	Blood Pb with lactational exposure: 46 µg/dL
	Increases in neuronal cell apoptosis found animals with Pb exposure	Section 4.3.9.5	

[a]Described in detail in Table II of the Preamble.

[b]Describes the key evidence and references contributing most heavily to causal determination and, where applicable, to uncertainties or inconsistencies. References to earlier sections indicate where full body of evidence is described.

[c]Describes the Pb biomarker levels with which the evidence is substantiated and blood Pb levels in animals most relevant to this ISA.

4.4 Cardiovascular Effects

4.4.1 Introduction

The 2006 Pb AQCD (U.S. EPA, 2006b) concluded that both epidemiologic and animal toxicological studies support the relationship between increased Pb exposure and increased cardiovascular effects, in particular, increased blood pressure (BP) and increased incidence of arterial hypertension. Although fewer in number, epidemiologic studies demonstrated associations of blood and bone Pb levels with other cardiovascular diseases (CVDs) in adults, such as ischemic heart disease, cerebrovascular disease, peripheral vascular disease, and CVD-related mortality. As the cardiovascular and renal systems are intimately linked, cardiovascular effects can arise secondarily to Pb-induced renal injury (Section 4.5). Toxicological studies also provided compelling evidence supporting the biological plausibility for Pb-associated cardiovascular effects by characterizing a number of the underlying mechanisms by which Pb exposure can lead to human cardiovascular health effects. Such studies demonstrated that the Pb content in heart tissue of animals reflects the increases in blood Pb levels (Lal et al., 1991), indicating that the cardiovascular morbidity associated with blood Pb levels may represent the effects of the bioavailable Pb in the target tissue. The strongest evidence supported the role of oxidative stress in the pathogenesis of Pb-induced hypertension. Additionally, several toxicological studies characterized other pathways or cellular, molecular, and tissue events promoting the Pb-induced increase in BP. These mechanisms included inflammation, adrenergic and sympathetic activation, renin-angiotensin-aldosterone system (RAAS) activation, vasomodulator imbalance, and vascular cell dysfunction.

With regard to the concentration-response relationship, a meta-analysis of human studies found that each doubling of blood Pb level (within a range extending from 1 at the lower end to above 40 μg/dL at the upper end, measured concurrently in most studies) was associated with a 1 mmHg increase in systolic BP and a 0.6 mmHg increase in diastolic BP (Nawrot et al., 2002). On a population-wide basis, the estimated effect size could translate into a clinically significant increase in the segment of the population with the highest BP. In a moderately-sized population, a relatively small effect size thus has important health consequences for the risk of sequelae of increased BP, such as stroke, myocardial infarction, and sudden death. It was also noted that most of the reviewed studies examining bone Pb levels, biomarkers of cumulative Pb exposure, also showed increased BP (Cheng et al., 2001; Hu et al., 1996a) or increased hypertension with

increasing bone Pb level (Lee et al., 2001a). Across studies, over a range of bone Pb concentrations (<1.0 to 96 μg/g), every 10 μg/g increase in bone Pb was associated with increased odds ratios of hypertension between 1.28 and 1.86. Studies observed an average increase in systolic BP of ~0.75 mmHg for every 10 μg/g increase in bone Pb concentration over a range of <1 to 52 μg/g.

With regard to etiologically-relevant timing of Pb exposure, toxicological evidence demonstrated increases in BP after long-term (>4 weeks) Pb exposure. In epidemiologic studies, cardiovascular outcomes were most often examined in cross-sectional studies with one or a limited number of Pb biomarker measurements, so uncertainty exists as to the specific Pb exposure level, timing, frequency, and duration that contributed to the observed associations. While associations of adult bone Pb (particularly tibia Pb) with health outcomes in adults are indicative of effects related to past or cumulative exposures, interpretation of similar associations involving adult blood Pb levels, especially those measured concurrently with outcomes, is complicated by the higher past exposures generally observed in U.S. adults populations. Detailed interpretation of Pb in blood and bone are provided in Sections 3.3 and 3.7.3. Briefly, higher past Pb exposures in adults increased their bone Pb stores which contribute to current blood Pb levels through the normal process of bone remodeling, as well as periods of increased bone remodeling and loss (e.g., osteoporosis and pregnancy). Due to the long latency period for the development of increased BP and CVD, associations of cardiovascular effects with low concurrent blood Pb levels (e.g., population means 1.6-4 μg/dL) in adults may be influenced by higher past Pb exposures (Section 3.4.1).

Past air Pb concentration and blood Pb data provide context for the cardiovascular studies. Section 2.2 notes that the peak U.S. use of Pb anti-knock additives in automobile gasoline and peak industrial emissions occurred between 1968 and 1972 and Pb in gasoline was finally banned from use in 1996. Section 2.5 shows that the annual average of the maximum 3-month average air Pb concentration for trends monitors across the U.S. decreased from 1.3 μg/m^3 in 1980 to 0.14 μg/m^3 in 2010. The mean 2010 3-month rolling average for non-source monitors (i.e., monitors not in close proximity to specific sources) was an order of magnitude lower than the 2010 trends site average. Studies of blood Pb during this time period of peak air Pb concentrations in the U.S. (1968-1980) show higher blood Pb levels than 2009-2010 levels presented in Section 3.4. Among subjects ages 1-74 years examined in the U.S. NHANES II study (1976-1980) by Pirkle et al. (1994), the geometric mean blood Pb level was 12.8 μg/dL. When stratified by age, studies report geometric mean blood Pb levels in adults (age 20-79 years in 1968-1980) of 13.1 – 16.2 μg/dL (Pirkle et al., 1994; Tepper and Levin, 1975) and in children (age 1 mo – 6 years in 1970-1980) of 15.0-30.4 μg/dL (Pirkle et al., 1994; Billick et al., 1979; Fine et al., 1972).

This section reviews the published studies pertaining to the cardiovascular effects of Pb exposure in humans, experimental animals, isolated vascular tissues, and cultured vascular cells. With the large and strong existing body of evidence serving as the foundation, emphasis was placed on studies published since the 2006 Pb AQCD. Epidemiologic and toxicological studies continued to augment the evidence for increases in BP and hypertension development associated with long-term Pb exposure and expanded the evidence for the biological pathways of these effects. Epidemiologic studies strengthened the evidence for associations between Pb biomarkers and cardiovascular effects after adjusting for potential confounding factors such as age, SES, diet, alcohol use, BMI, comorbidities, and smoking. Emphasis was placed on studies that had extensive consideration for confounding and prospective study designs. The epidemiologic evidence was substantiated with results from several available prospective studies demonstrating the directionality of effects by indicating associations between Pb biomarkers and the subsequent incidence of cardiovascular health effects.

4.4.2 Blood Pressure and Hypertension

4.4.2.1 Epidemiology

The most commonly used indicator of cardiovascular morbidity was increased BP and its derived index, hypertension. Hypertension in these studies was defined as diastolic and/or systolic BP above certain cut-points or use of anti-hypertensive medicines. The BP cut-points were established by reference to informed medical opinion, but BP cut-points defining hypertension have been lowered over time, as medical knowledge has improved. Consequently, different studies using "hypertension" as a cardiovascular outcome may have assigned different cut-points, depending on the year and location of the study and the individual investigator. All of the recent studies in the current review used similar criteria for hypertension (e.g., systolic BP at or above 140, diastolic BP at or above 90, or use of anti-hypertensive medications). Studies in the medical literature show that elevated BP is associated with increased risk of CVD including coronary disease, stroke, peripheral artery disease, and cardiac failure. Coronary disease (i.e., myocardial infarction, angina pectoris, and sudden death) is the most lethal sequelae of hypertension (Ingelsson et al., 2008; Chobanian et al., 2003; Pastor-Barriuso et al., 2003; Prospective Studies Collaboration, 2002; Kannel, 2000a, b; Neaton et al., 1995).

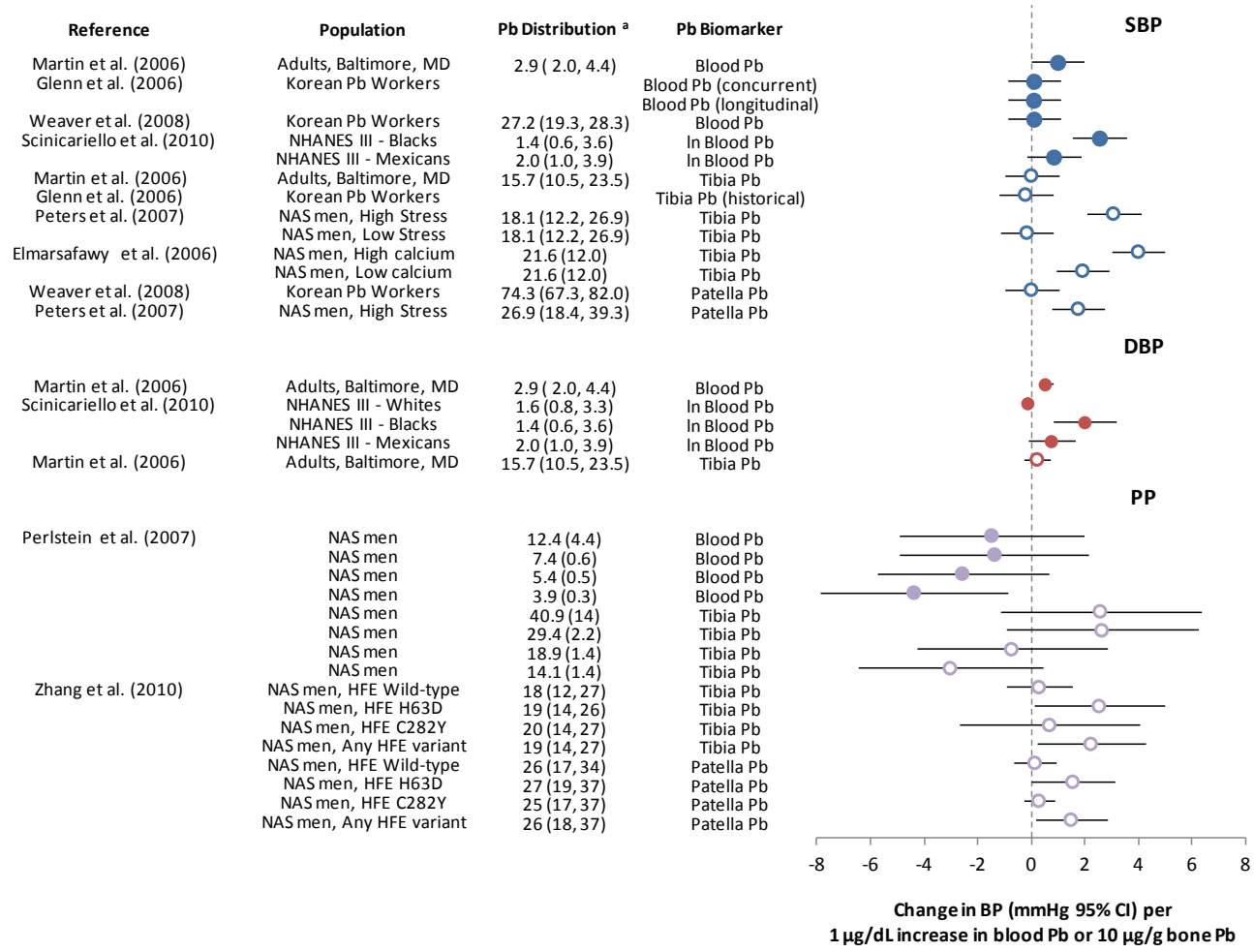
Earlier, U.S. EPA (1990a) reviewed the then available studies examining Pb exposure and BP and hypertension outcomes which included evaluation of several studies conducting analysis of the data in NHANES II (1976-80). They noted that across a range

of 7 to 34 µg/dL, no evident threshold was found below which concurrent blood Pb was not significantly related to BP. U.S. EPA (1990a) concluded that a small but positive association exists between blood Pb levels and increases in BP. Quantitatively, the relationship appeared to hold across a wide range of blood Pb values and, furthermore, an estimated mean increase of 1.5-3.0 mmHg in systolic BP appeared to occur for every doubling of blood Pb concentration in adult males and less than 1.0-2.0 mmHg for adult females. U.S. EPA (1990a) further concluded that the plausibility of these relationships observed in epidemiologic studies of human populations being of a causal nature is supported by controlled experimental animal studies demonstrating increased BP clearly attributable to Pb. Subsequently, the 2006 Pb AQCD (U.S. EPA, 2006b) reviewed the literature examining Pb exposure and effects of BP and hypertension, published after the 1990 document as discussed in Section 4.4.1.

Several recent general population and occupational cohort and cross-sectional studies strengthened the evidence for associations of blood and bone Pb levels with measures of BP (Figure 4-17 and Table 4-18) and with the prevalence and incidence of hypertension (Figure 4-18 and Table 4-19). Further, recent studies expanded evidence, finding differences in association among racial/ethnic groups, perceived stress, diet, and genetic variants, and thus, identified populations potentially at increased risk of Pb-associated cardiovascular effects.

In a cross-sectional analysis, Martin et al. (2006) examined the associations of concurrent blood and tibia Pb levels with BP and hypertension in a large, community-based study of older adults (N = 964, age ranging from 50 to 70 years) in Baltimore, MD. Although cross-sectional in design, a key strength of this study was the extensive consideration of potential confounding variables. Four models evaluated associations for BP and hypertension. The base model included age, sex, BMI, sodium intake, potassium intake, total cholesterol, time of day, testing technician, and hypertensive medication use. Other models added SES, race/ethnicity, or both as covariates. Blood Pb but not tibia Pb level was a strong predictor of BP in all models; a 1 µg/dL increase in concurrent blood Pb level was associated with an approximately 1 mmHg increase in systolic BP and an approximately 0.5 mmHg increase in diastolic BP. Tibia Pb but not blood Pb was associated with hypertension in logistic regression models. The authors applied propensity analysis to their models to better account for the effect of other risk factors for hypertension such as race/ethnicity, age, and SES that were strongly associated with tibia Pb level. The propensity score analysis and model adjustment did not substantially change the numerical findings and conclusions (e.g., tibia Pb and hypertension were positively associated independently of race/ethnicity and SES), indicating that neither SES nor race/ethnicity confounded the association between tibia Pb level and hypertension. No evidence for effect modification by race/ethnicity was found either.

Martin et al. (2006) concluded that Pb in blood has a short term effect on BP and that Pb contributes to hypertension risk as a function of cumulative, chronic exposure (as represented as bone Pb in this population). While different aspects of Pb exposure may contribute differentially to increases in BP and hypertension, it is important to note that concurrent blood Pb levels in adults also reflect cumulative Pb exposure. Thus, its association with BP may not reflect a short term effect but may instead reflect an effect of cumulative Pb exposure.



[a]Pb distributions present the median (IQR), which were estimated from the mean and SD assuming a normal distribution.

[b]Effect estimates were standardized to 1 µg/dL blood Pb or 10 µg/g bone Pb.

Note: In general, results are categorized by specific BP parameter, then by Pb biomarker. For categories with multiple studies, the order of the studies follows the order of discussion in the text. Results display associations (95% CI) of a 1 µg/dL increase in blood Pb level (closed circles) or 10 µg/g increase in bone Pb (open circles) with systolic BP (SBP; blue), diastolic BP (DBP; red), and pulse pressure (PP; purple) in adults.

Figure 4-17 Associations of blood and bone Pb levels with systolic BP, diastolic BP, and pulse pressure in adults.

Table 4-18 Additional characteristics and quantitative data for associations of blood and bone Pb with BP measures for studies presented in Figure 4-17.

Study	Study Population / Methodology	Parameter	Pb Data	Statistical Analysis	Effect Estimate β (95% CI)
Martin et al. (2006)	Cross-sectional 964 men and women, ages 50-70 yr, 40% African American, 55% White, 5% other, in Baltimore, MD	BP	Concurrent Mean Blood Pb: Mean (SD): 3.5 (2.3) µg/dL African American: 3.4 (2.3) White: 3.5 (2.4) Tibia Pb: Mean (SD): 18.8 (12.4) µg/g African American: 21.5 (12.6) White: 16.7 (11.9)	Extensive analysis of potential confounding factors. Multiple linear regression base models adjusted for age, sex, BMI, antihypertensive medication use, dietary sodium intake, dietary potassium intake, time of day, testing technician, serum total cholesterol. SES, race/ ethnicity also included in models that are presented in Figure 4-17, and tabulated here.)	Blood Pb: SBP: 1.05 (0.53,1.58) DBP: 0.53 (0.25, 0.81) mmHg per µg/dL blood Pb Tibia Pb: SBP: 0.07 (-0.05, 0.14) DBP: 0.05 (-0.02, 0.08) mmHg per µg/g bone Pb
Glenn et al. (2006)	Longitudinal 575 Pb exposed workers, ages 18-65 yr, in South Korea (10/1997-6/2001)	BP	Blood Pb mean (SD): Visit 1: 20.3 (9.6), Women Visit 2: 20.8 (10.8), Women Visit 3: 19.8 (10.7), Women Visit 1: 35.0 (13.5), Men Visit 2: 36.5 (14.2), Men Visit 3: 35.4 (15.9), Men	Multivariable models using GEE were used in longitudinal analyses. Models were adjusted for visit number, baseline age, baseline age squared, baseline lifetime alcohol consumption, baseline BMI, sex, baseline BP lowering medication use, alcohol consumption.	Model 1 (short-term) Blood Pb (longitudinal): 0.09 (0.01, 0.16) Blood Pb (concurrent): 0.08 (-0.01, 0.16) Model 4 (short and longer-term) Blood Pb (longitudinal): 0.09 (0.01, 0.16) Blood Pb (concurrent): 0.10 (0.01, 0.19) mmHg per 10 µg/dL blood Pb

Table 4-18 (Continued): Additional characteristics and quantitative data for associations of blood and bone Pb with BP measures for studies presented in Figure 4-17.

Study	Study Population / Methodology	Parameter	Pb Data	Statistical Analysis	Effect Estimate β (95% CI)
Weaver et al. (2008)	Cross-sectional 652 current and former Pb workers in South Korea (12/1999-6/2001) Same cohort as Glenn et al. (2006)	BP	Concurrent Blood Pb: Mean (SD): 30.9 (16.7) µg/dL Concurrent Patella Pb: Mean (SD): 75.1 (101.1) µg/g	Linear regression model adjusted for age, sex, BMI, diabetes, antihypertensive and analgesic medication use, Pb job duration, work status, tobacco and alcohol use	SBP Patella Pb: 0.0059 (-0.008, 0.02)[a] Blood Pb: 0.1007 (0.02, 0.18)[a] mmHg per 1 µg/dL blood Pb or 1 µg/g patella Pb Interaction between blood Pb/patella Pb with ALAD and vitamin D receptor polymorphisms not significant.
Perlstein et al. (2007)	Cross-sectional 593 elderly men (mostly white) enrolled in NAS in Greater Boston, MA area (1991-1997)	PP	Blood Pb: Overall mean (SD): 6.12 (4.03) µg/dL Quintile means (SD): Q1: 2.3 (0.8) µg/dL Q2: 3.9 (0.3) µg/dL Q3: 5.4 (0.5) µg/dL Q4: 7.4 (0.6) µg/dL Q5: 12.4 (4.4) µg/dL Tibia Pb: Overall median: 19 µg/g Quintile means (SD): Q1: 7.4 (3.2) µg/g Q2: 14.1 (1.4) µg/g Q3: 18.9 (1.4) µg/g Q4: 24.9 (2.2) µg/g Q5: 40.9 (14) µg/g	BP association assessed using Spearman correlation coefficients. PP association (adjusted mean difference) assessed using multiple linear regression model adjusted for age, height, race, heart rate, waist circumference, diabetes, family history of hypertension, education level achieved, smoking, alcohol intake, fasting plasma glucose, and ratio of total cholesterol to HDL cholesterol	PP 4.2 (1.9, 6.5) mmHg higher in men with tibia Pb >19 µg/g (median) compared with men with tibia Pb below the median Blood Pb (mean difference): Q5: -1.49 (–4.93, 1.94) Q4: -1.39 (–4.94, 2.15), Q3: -2.56 (–5.78, 0.67) Q2: -4.37 (–7.88,–0.86) Q1: Referent group Tibia Pb (mean difference): Q5: 2.58 (–1.15, 6.33) Q4: 2.64 (–0.93, 6.21) Q3: -0.73 (–4.27, 2.82) Q2: -3.02 (–6.48, 0.44) Q1: Referent group

Table 4-18 (Continued): Additional characteristics and quantitative data for associations of blood and bone Pb with BP measures for studies presented in Figure 4-17.

Study	Study Population / Methodology	Parameter	Pb Data	Statistical Analysis	Effect Estimate β (95% CI)
Peters et al. (2007)	Longitudinal and Cross-sectional 513 elderly men (mostly white, mean age 67 yr) enrolled in NAS in Greater Boston, MA area	BP	Tibia Pb: mean (SD): 21.5 (13.4) µg/g Patella Pb: Mean (SD): 31.5 (19.3) µg/g	Logistic and linear regression models adjusted for age, age squared, sodium, potassium, and Ca^{2+} intake, family history of hypertension, BMI, educational level, pack-years of smoking, alcohol consumption, and physical activity	SBP Tibia Pb/ High Stress: 3.57 (0.39, 6.75) Tibia Pb/ Low Stress: 0.21 (-1.70, 1.29) per SD increase in tibia Pb Patella Pb/ High Stress: 2.98 (-0.12, 6.08) per SD increase in patella Pb Patella Pb/ Low Stress: NR
Elmarsafawy et al. (2006)	Cross-sectional 471 elderly men (mostly white, mean age 67 yr) enrolled in NAS in Greater Boston, MA area	BP	Blood Pb: Mean (SD): 6.6 (4.3) µg/dL Tibia Pb: Mean (SD): 21.6 (12.0) µg/g Patella Pb: Mean (SD): 31.7 (18.3) µg/g	Linear regression models adjusted for age, BMI, family history of hypertension, history of smoking, dietary sodium intake, and cumulative alcohol ingestion Lack of consideration for potential confounding by SES-related variables.	SBP Tibia Pb High Ca^{2+} group (>800 mg/day): 0.40.(0.11, 0.70) Low Ca^{2+} group (<800 mg/day): 0.19 (0.01, 0.37) mmHg per µg/g tibia Pb

Table 4-18 (Continued): Additional characteristics and quantitative data for associations of blood and bone Pb with BP measures for studies presented in Figure 4-17.

Study	Study Population / Methodology	Parameter	Pb Data	Statistical Analysis	Effect Estimate β (95% CI)
Zhang et al. (2010a)	Cross-sectional 619 elderly men (mostly white, mean age 67 yr) enrolled in the NAS in Greater Boston, MA area	PP	Wild type HFE Tibia Pb: Median (IQR): 8 (12-27) µg/g Patella Pb: Median (IQR): 26 (17-37) µg/g C282Y HFE Tibia Pb: Median (IQR): 20 (14-27) µg/g Patella Pb: Median (IQR): 25 (17-37) µg/g H63D HFE Tibia Pb: Median (IQR): 19 (14-26) µg/g Patella Pb: Median (IQR): 27 (19-37) µg/g	Linear mixed effects regression models with repeated measurements adjusted for age; education; alcohol intake; smoking; daily intakes of Ca^{2+}, sodium, and potassium; total calories; family history of hypertension; diabetes; height; heart rate; high-density lipoprotein (HDL); total cholesterol:HDL ratio; and waist circumference	PP mmHg per 13 µg/g Tibia Pb: Wild Type HFE: 0.38 (0,1.96) H63D HFE: 3.30 (0.16, 6.46) C282Y HFE: 0.89 (0, 5.24) Any HFE variant: 2.90 (0.31, 5.51) mmHg per 19 µg/g Patella Pb: Wild Type HFE: 0.26 (0, 1.78) H63D HFE: 2.95 (0, 5.92) C282Y HFE: 0.55 (0, 1.66) Any HFE variant: 2.83 (0.32,5.37)

Table 4-18 (Continued): Additional characteristics and quantitative data for associations of blood and bone Pb with BP measures for studies presented in Figure 4-17.

Study	Study Population / Methodology	Parameter	Pb Data	Statistical Analysis	Effect Estimate β (95% CI)
Scinicariello et al. (2010)	Cross-sectional 6,016 NHANES III (1988-1994) participants age ≥ 17 yr	BP	Concurrent Blood Pb: Mean (SE): Overall: 2.99 (0.09) µg/dL Non-Hispanic Whites: 2.87 (0.09) Non-Hispanic Blacks 3.59 (0.20) Mexican American 3.33 (0.11)	Multivariable linear regression of log-transformed blood Pb level adjusted for age, sex, education, smoking status, alcohol intake, BMI, serum creatinine levels, serum Ca^{2+}, glycosylated hemoglobin, and hematocrit	SBP Non-Hispanic whites: 1.05 (0.32, 1.78) Non-Hispanic blacks: 2.55 (1.59, 3.51) Mexican Americans: 0.84 (-0.06, 1.74) DBP Non-Hispanic whites: -0.14 (-1.1, 0.82) Non-Hispanic blacks: 1.99 (1.13, 2.85) Mexican Americans: 0.74 (-0.005, 1.48) mmHg per unit increase in logarithmic (ln)-transformed blood Pb Significant interactions with blood Pb and ALAD genotype observed in relation to SBP for non-Hispanic whites and non-Hispanic blacks

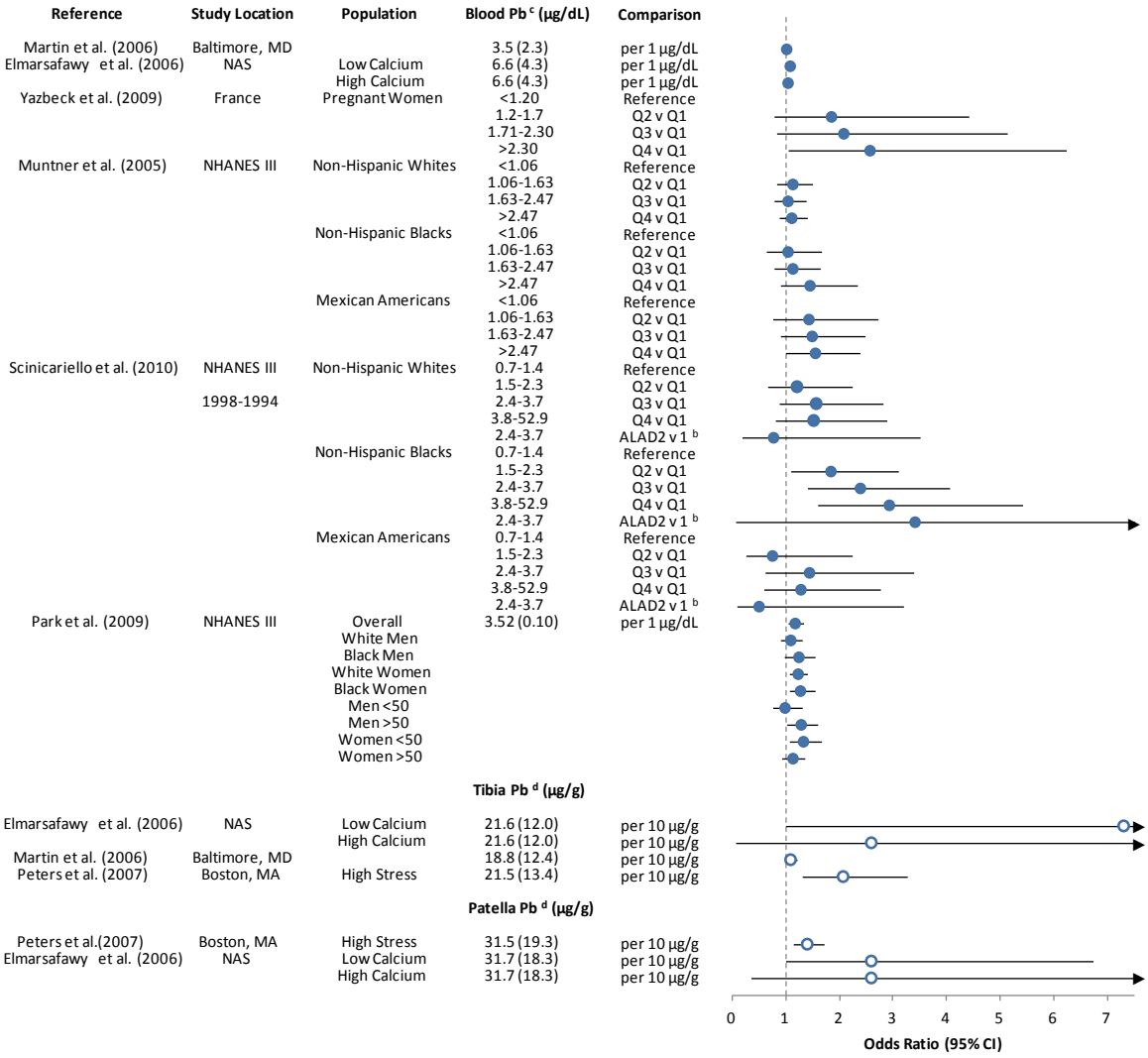
Table 4-18 (Continued): Additional characteristics and quantitative data for associations of blood and bone Pb with BP measures for studies presented in Figure 4-17.

Study	Study Population / Methodology	Parameter	Pb Data	Statistical Analysis	Effect Estimate β (95% CI)
Navas-Acien et al. (2008)[b]	Longitudinal and Cross-sectional Meta-analysis of studies using bone Pb as an exposure metric and BP as the outcome (8 studies)	BP	-	Inverse variance weighted random-effects meta-analyses	BP Pooled Estimates mmHg per 10 µg/g Tibia Pb Prospective SBP 0.33 (-0.44, 1.11) Cross-sectional SBP 0.26 (0.02, 0.50) Cross-sectional DBP 0.02 (-0.15, 0.19) Hypertension per 10 µg/g patella Pb Cross-sectional hypertension OR: 1.04 (1.01, 1.07) Meta-analysis estimate hypertension OR: 1.04 (0.96, 1.12)
Yazbeck et al. (2009)[c]	Cross-sectional 971 pregnant women, ages 18-45 yr, in France	BP	Midpregnancy Blood Pb: PIH group mean (SD): 2.2 (1.4) No PIH group mean (SD): 1.9 (1.2)	Multivariable logistic regression models adjusted for maternal age; Cd, Mn, and Se blood levels; hematocrit; parity; BMI; pregnancy weight gain; gestational diabetes; educational level; SES; geographic residence; and smoking status and alcohol consumption before and during pregnancy	Log-transformed blood Pb at mid-pregnancy SBP: r = 0.08; p = 0.03 DBP: r = 0.07; p = 0.03 Significant correlations also observed after 24 weeks of gestation and after 36 weeks of gestation.

[a]95% CIs estimated from given p-value.

[b]Reference not included in Figure 4-17, because it is a meta-analysis.

[c]Reference not included in Figure 4-17, because only correlations were reported



Note: Studies are categorized by Pb biomarker (blood Pb [closed circles] or bone Pb [open circles]) for hypertension prevalence and incidence[a]. Within each category, studies generally are presented in order of discussion in the text.

[a]The outcomes plotted are hypertension prevalence with the exception of Yazbeck et al. (2009) which measured pregnancy induced hypertension and Peters et al. (2007) which measured hypertension incidence.

[b]b: ALAD2 vs. 1 indicates comparison between ALAD 2 carriers (e.g., ALAD1-2 and ALAD2-2) and ALAD 1 homozygotes (e.g., ALAD1-1).

[c]c: Effect estimates were standardized to a 1 µg/dL increase in blood Pb (closed circles).

[d]d: Effect estimates were standardized to a 10 µg/g increase in bone Pb (open circles).

Figure 4-18 Odds ratios (95% CI) for associations of blood Pb and bone Pb with hypertension prevalence and incidence.

Table 4-19 Additional characteristics and quantitative data for results presented in Figure 4-18 for associations of blood and bone Pb with hypertension measures.

Study (Ordered as they appear in the text)	Study Population and Methodology	Parameter	Pb Data	Statistical Analysis	Effect Estimate (95% CI)
Martin et al. (2006)	Cross-sectional 964 men and women, ages 50-70 yr, 40% African American, 55% White, 5% other, in Baltimore, MD	Hypertension (current use of antihypertensive medication, mean SBP ≥ 140 mmHg or DBP ≥ 90 mmHg)	Blood Pb: Mean (SD): 3.5 (2.3) µg/dL Tibia Pb: Mean (SD): 18.8 (12.4) µg/g	Logistic regression models adjusted for age, sex, BMI, antihypertensive medication use, dietary sodium intake, dietary potassium intake, time of day, testing technician, and serum homocysteine Lack of consideration for potential confounding by SES-related variables.	Blood Pb level: OR=1.02 (0.87, 1.19) Tibia Pb: OR=1.24 (1.05, 1.47)
Weaver et al. (2008)[a]	Cross-sectional 652 current and former Pb workers in South Korea (12/1999-6/2001)	Hypertension (mean SBP ≥ 140 mmHg, DBP ≥ 90 mmHg; and/or use of antihypertensive medications; or physician diagnosis)	Blood Pb: Mean (SD): 31.9 (14.8) µg/dL Patella Pb: Mean (SD): 37.5 (41.8) µg/g	Logistic regression models adjusted for age, sex, BMI, diabetes, antihypertensive and analgesic medication use, Pb job duration, work status, tobacco and alcohol use	Quantitative results not reported. None of the examined Pb exposure metrics (blood, patella, and logarithmic (ln)-transformed patella) were significantly associated with hypertension
Peters et al. (2007)	Longitudinal 513 elderly men (mostly white, mean age 67 yr) enrolled in NAS in Greater Boston, MA area	Hypertension (mean SBP >140 mmHg, DBP >90 mmHg; or physician diagnosis)	Tibia Pb: Mean (SD): 21.5 (13.4) µg/g Patella Pb: Mean (SD): 31.5 (19.3) µg/g	Cox proportional hazards models adjusted for age, age squared, sodium, potassium, and Ca^{2+} intake, family history of hypertension, BMI, educational level, smoking, alcohol consumption, baseline SBP and DBP, and physical activity	Risk of Hypertension Incidence High Stress RR=2.66 (1.43, 4.95) per SD increase in tibia Pb RR=2.64 (1.42, 4.92) per SD increase in patella Pb

Table 4-19 (Continued): Additional characteristics and quantitative data for results presented in Figure 4-18 for associations of blood and bone Pb with hypertension measures.

Study (Ordered as they appear in the text)	Study Population and Methodology	Parameter	Pb Data	Statistical Analysis	Effect Estimate (95% CI)
Elmarsafawy et al. (2006)	Cross-sectional 471 elderly men (mostly white, mean age 67 yr) enrolled in NAS in Greater Boston, MA area	Hypertension (mean SBP ≥ 160 mmHg, DBP ≥ 95 mmHg; and/or physician diagnosis with current use of antihypertensive medications)	Blood Pb: Mean (SD): 6.6 (4.3) µg/dL Tibia Pb: Mean (SD): 21.6 (12.0) µg/g Patella Pb: Mean (SD): 31.7 (18.3) µg/g	Logistic regression models adjusted for age, BMI, family history of hypertension, history of smoking, dietary sodium intake, and cumulative alcohol ingestion	Low Ca^{2+} group (<800 mg/day): Blood Pb OR: 1.07 (1.00, 1.15) Tibia Pb OR: 1.02 (1.00, 1.04) Patella Pb OR: 1.01 (1.00, 1.03) High Ca^{2+} group (>800 mg/day): Blood Pb OR: 1.03 (0.97, 1.11) Tibia Pb OR: 1.01 (0.97, 1.04) Patella Pb OR: 1.01 (0.99, 1.03) Per µg/dL blood Pb or µg/g tibia or patella Pb
Yazbeck et al. (2009)[a]	Cross-sectional 971 pregnant women, ages 18-45 yr, in France	PIH (SBP ≥ 140 mmHg or DBP ≥ 90 mmHg after the 22nd week of gestation)	Blood Pb (µg/dL) PIH group mean (SD): 2.2 (1.4) No PIH group mean (SD): 1.9 (1.2) Q1: <1.20 Q2: 1.20-1.70 Q3: 1.71-2.30 Q4: >2.30	Multivariable logistic regression models adjusted for maternal age, Cd, Mn, and Se blood levels, parity, hematocrit, BMI, gestational diabetes, educational levels, SES, geographic residence, and smoking status during pregnancy	PIH Blood Pb OR=3.29 (1.11, 9.74) per 1 unit increase in log maternal blood Pb level Q1: Reference group Q2: OR=1.84 (0.77, 4.41) Q3: OR=2.07 (0.83, 5.13) Q4: OR=2.56 (1.05, 6.22)

Table 4-19 (Continued): Additional characteristics and quantitative data for results presented in Figure 4-18 for associations of blood and bone Pb with hypertension measures.

Study (Ordered as they appear in the text)	Study Population and Methodology	Parameter	Pb Data	Statistical Analysis	Effect Estimate (95% CI)
Muntner et al. (2005)	Cross-sectional 9,961 NHANES (1999-2002) participants, ages ≥18 yr	Hypertension (current use of antihypertensive medication, SBP ≥ 140 mmHg, or DBP ≥ 90 mmHg)	Concurrent Blood Pb (µg/dL) Overall Mean (95% CI): 1.64 (1.59-1.68) Q1: <1.06 Q2: 1.06-1.63 Q3: 1.63-2.47 Q4:≥ 2.47	Multivariable logistic regression models adjusted for age, sex, diabetes mellitus, BMI, cigarette smoking, alcohol consumption, high school education, and health insurance status Monotonic increase in OR across blood Pb level groups.	Non-Hispanic white: Q1: Reference group Q2: OR=1.12 (0.83, 1.50) Q3: OR=1.03 (0.78, 1.37) Q4: OR=1.10 (0.87, 1.41) Non-Hispanic black Q1: Reference group Q2: OR=1.03 (0.63, 1.67) Q3: OR=1.12 (0.77, 1.64) Q4: OR=1.44 (0.89, 2.32) Mexican American Q1: Reference group Q2: OR=1.42 (0.75, 2.71) Q2: OR=1.48 (0.89, 2.48) Q3: OR=1.54 (0.99, 2.39) p for trend=0.04

Table 4-19 (Continued): Additional characteristics and quantitative data for results presented in Figure 4-18 for associations of blood and bone Pb with hypertension measures.

Study (Ordered as they appear in the text)	Study Population and Methodology	Parameter	Pb Data	Statistical Analysis	Effect Estimate (95% CI)
Scinicariello et al. (2010)	Cross-sectional 6,016 NHANES III (1988-1994) participants, ages ≥ 17 yr	Hypertension (current use of antihypertensive medication, SBP ≥ 140 mmHg, or DBP ≥ 90 mmHg)	Concurrent Blood Pb (µg/dL) Mean (SE): 2.99 (0.09) Q1: 0.7-1.4 Q2: 1.5-2.3 Q3: 2.4-3.7 Q4: 3.8-52.9 Mean (SE): Non-Hispanic Whites: 2.87 (0.09) Non-Hispanic Blacks: 3.59 (0.20) Mexican American: 3.33 (0.11)	Multivariable logistic regression model adjusted for race/ethnicity, age, sex, education, smoking status, alcohol intake, BMI, serum creatinine levels, serum Ca^{2+}, glycosylated hemoglobin, and hematocrit	Non-Hispanic whites: Q1: Reference group Q2: POR=1.21 (0.66, 2.24) Q3: POR=1.57 (0.88, 2.80) Q4: POR=1.52 (0.80, 2.88) ALAD1-2/2-2: POR= 0.76 (0.17, 3.50) ALAD-1: Reference group Non-Hispanic blacks: Q1: Reference Q2: POR=1.83 (1.08, 3.09) Q3: POR=2.38 (1.40, 4.06) Q4: POR=2.92 (1.58, 5.41) ALAD1-2/2-2: POR= 3.40 (0.05, 219.03) ALAD-1: Reference group Mexican Americans: Q1: Reference Q2: POR=0.74 (0.24, 2.23) Q3: POR=1.43 (0.61, 3.38) Q4: POR=1.27 (0.59, 2.75) ALAD1-2/2-2: POR= 0.49 (0.08, 3.20) ALAD-1: Reference group ALAD2 carriers in non-Hispanic white population in highest blood Pb quartile with blood Pb level <10 µg/dL associated with hypertension compared to ALAD1 homozygous individuals (POR=1.86 [1.00, 3.49]).

Table 4-19 (Continued): Additional characteristics and quantitative data for results presented in Figure 4-18 for associations of blood and bone Pb with hypertension measures.

Study (Ordered as they appear in the text)	Study Population and Methodology	Parameter	Pb Data	Statistical Analysis	Effect Estimate (95% CI)
Park et al. (2009b)	Cross-sectional 12,500 NHANES III (1988-1994) participants, mean age 49.9 yr	Hypertension	Concurrent Blood Pb Mean and SE (μg/dL) Overall: 3.52 (0.10) White men <50 yr: 4.02 (0.16) ≥ 50 yr: 4.92 (0.18) Black men <50 yr: 4.55 (0.15) ≥ 50 yr: 7.57 (0.22) White women <50 yr: 2.09 (0.07) ≥ 50 yr: 3.53 (0.12) Black women <50 yr: 2.52 (0.09) ≥ 50 yr: 4.49 (0.16)	Logistic regression models adjusted for age, education, smoking status, cigarette smoking, BMI, hematocrit, alcohol consumption, physical activity, antihypertensive medication use, and diagnosis of type-2 diabetes	OR per SD (0.75 μg/dL) in log blood Pb: Overall: 1.12 (1.03, 1.23). White men: 1.06 (0.92, 1.22) Black men: 1.17 (0.98, 1.38) White women: 1.16 (1.04, 1.29) Black women: 1.19 (1.04, 1.38) Men <50 yr: 0.98 (0.80, 1.22) Men >50 yr: 1.20 (1.02, 1.41) Women <50 yr: 1.23 (1.04, 1.46) Women >50 yr: 1.09 (0.94, 1.26)

[a]Not included in Figure 4-18 because OR data were not reported.

In an occupational cohort in South Korea, Glenn et al. (2006) simultaneously modeled multiple Pb dose measures of individuals collected repeatedly over four years of follow up. Thus, through the assessment of cross-sectional and longitudinal relationships with BP, this study provided key insight on potentially important time periods of Pb exposure and also informed the directionality of association. The initial blood Pb level was used as a baseline covariate and the difference in blood Pb level between visits was computed for each subsequent visit. The bone Pb measures (tibia Pb at visits 1 and 2, patella Pb at visit 3) were used to indicate historical exposure and cumulative dose. Four models were specified: Model 1 was conceptualized to reflect short-term changes in BP associated with recent dose; Model 2 to reflect longer-term changes associated with cumulative dose controlling for the association of baseline BP with recent dose; Model 3 to reflect longer-term changes associated with cumulative dose controlling for cross-sectional influence of cumulative dose on baseline BP; and Model 4 to reflect both short-term change with recent dose and longer-term change with cumulative dose. Concurrent blood Pb and increases in blood Pb between visits were associated with increases in systolic BP in Model 1 (short-term dose) and Model 4 (short- and longer-term dose). No association was observed between BP and tibia Pb at baseline while higher tibia Pb was associated with a decrease in systolic BP in each of the models.

Glenn et al. (2006) was strengthened by the analysis of associations between changes in blood Pb and changes in BP over time within individual subjects. These results indicate that circulating Pb (e.g., blood Pb) may act continuously on systolic BP and reductions in blood Pb may contribute to reductions in BP, while cumulative Pb exposure (represented by bone Pb in this study) may contribute to hypertension incidence by different mechanisms over longer time periods and in older subjects. This analysis in subjects (mean [SD] age at baseline 41.4 [9.5] years) with a low prevalence of hypertension suggests at least one of the biological pathways that influences how systolic BP responds to Pb operates over a relatively rapid timeframe. This may reflect an immediate response to Pb at a biochemical site of action as a consequence of the biologically available Pb circulating in blood. A persistent effect of cumulative doses over a lifetime may occur via other mechanisms. Bone Pb level may exert influence on blood Pb levels and consequently on BP in an aging population with prolonged Pb exposure. Thus, the findings contribute important information regarding the various short and long-term exposure relationships with increases in BP and hypertension. It is important to acknowledge the uncertainty regarding the applicability of these findings regarding short-term and long-term effects in Pb workers with relatively high current Pb exposures contributing to blood Pb levels (mean blood Pb levels over time: 20-37 µg/dL) to adults in the U.S. general population whose concurrent blood Pb levels are influenced more by Pb mobilized from bone stores. Further, for bone Pb analysis, the potential for bone Pb, BP, and hypertension findings to be impacted by residual confounding by age may be a

factor to consider in older populations since exposure studies of older cohorts (NAS/ mean age >60 years; (Wilker et al., 2011; Kim et al., 1997)) indicate that bone Pb is correlated with age.

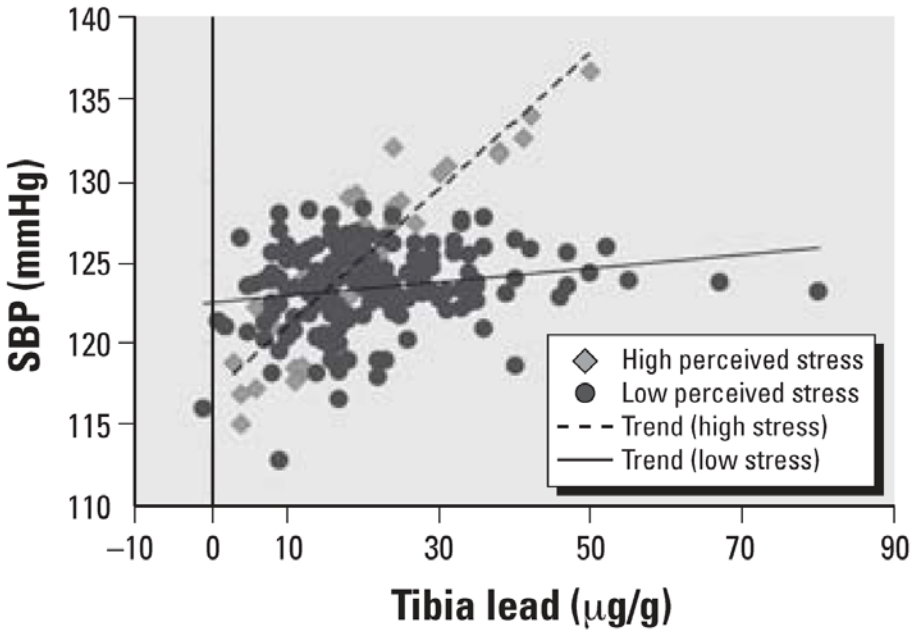
In a separate cross-sectional analysis of the same occupationally-exposed group in year three of follow-up, Weaver et al. (2008) examined associations of concurrent patella Pb and blood Pb level with systolic BP, diastolic BP, and hypertension and effect modification by ALAD (the genotypes have different affinities for Pb) and vitamin D receptor (VDR) polymorphisms. None of the Pb biomarkers were associated with diastolic BP. Patella Pb alone was not significantly associated with systolic BP. However, blood Pb, either alone or with patella Pb, was significantly associated with higher systolic BP. The patella Pb-age and blood Pb-age interactions were not statistically significant. There were no significant associations of blood Pb or patella Pb with hypertension status or effect modification by age or sex. Further, interactions between polymorphisms of the VDR and of ALAD variants with blood Pb and patella Pb on systolic BP were not statistically significant. Mean blood Pb level was high (30.9 µg/dL) compared to non-occupational groups.

Weaver et al. (2010) provided the results of further analysis of this Korean worker cohort, with a focus on determining the functional form of the concentration-response relationships. In a log linear model, the coefficient indicated that every doubling of blood Pb level was associated with a systolic BP increase of 1.76 mmHg. The J test, a statistical test for determining which, if either, of two functional forms of the same variable provides a superior fit to data in non-nested models (Davidson and MacKinnon, 1981), returned a p-value of 0.013 in favor of the natural log blood Pb level over the linear blood Pb level specification. This analysis indicates that the systolic BP increase in this cohort is better described as a logarithmic function of blood Pb level within the range of the study than by a linear function.

Several analyses in the NAS cohort of predominantly white older men in the greater Boston area found associations of blood and bone Pb level with BP and hypertension, and they indicated effect modification by calcium intake, perceived stress, and HFE gene variants. In a cross-sectional analysis, Perlstein et al. (2007) found a statistically significant association between blood Pb and diastolic BP in adjusted models. The subjects in this study had at least one bone Pb measurement during the years 1991-1997 and were not on antihypertensive medication at the time of the measurement. While tibia Pb was not significantly associated with BP, it was associated with pulse pressure (PP). Men with tibia Pb above the median (19 µg/g) had a higher mean PP (4.2 mmHg [95% CI: 1.9, 6.5]) compared to men with tibia Pb below the median. The trend toward increasing PP with increasing quintile of tibia Pb was statistically significant although

none of the confidence intervals for PP referenced to the lowest quintile of tibia Pb (<7.4 μg/g) excluded the null value.

Peters et al. (2007) examined cross-sectionally the modification of the associations of tibia and patella Pb with BP and hypertension by self-reported stress (assessed by questionnaire) in NAS men. High stress also has been linked with higher BP, potentially via mechanisms also affected by Pb exposure including activation of sympathetic pathways, ROS, and the HPA axis. Among all subjects, higher bone Pb level was associated (statistically nonsignificant) with greater odds of hypertension status and higher systolic BP. As indicated in Figure 4-19, the association between systolic BP and tibia Pb differed between those with high and low self-reported stress (β for tibia Pb x stress interaction = 3.77 mmHg [95% CI: 0.46, 7.09]) per SD increase in tibia Pb. Stress also was found to modify the patella Pb-BP association (β for patella Pb x stress interaction = 2.60 mmHg [95% CI: -0.95, 6.15] per SD increase in patella Pb). Neither bone, self-reported stress, nor their interaction was associated significantly with diastolic BP. Peters et al. (2007) also used Cox proportional hazards models to assess the interaction of stress and bone Pb level in the development of hypertension among those free of hypertension at baseline. The results of this analysis showed that increasing tibia and patella Pb were associated with greater risk of developing hypertension among those with high stress compared to those with lower perceived stress (RR of developing hypertension among those with high stress: 2.66 [95% CI: 1.43, 4.95] per SD increase in tibia Pb and 2.64 [95% CI: 1.42, 4.92] per SD increase in patella Pb). These results provide evidence supporting adults with higher stress as a population potentially at increased risk of Pb-associated cardiovascular effects. Earlier, Cheng et al. (2001) examined the NAS cohort in 474 subjects without hypertension (mean [SD] blood Pb level: 5.87 [4.01]) at baseline measurement and analyzed linear models with patella Pb and reported that only patella Pb level was associated with a significant increase in the rate ratio for hypertension using a Cox's proportional hazards model.



Source: Peters et al. (2007)

Figure 4-19 The relationship between tibia Pb and estimated systolic BP (SBP) for those with high self-reported stress versus those with low self-reported stress.

Elmarsafawy et al. (2006) examined the modification of the relationship between Pb and hypertension by dietary calcium, with 467 subjects from the NAS. Responses on a semi-quantitative dietary frequency questionnaire with one-year recall were used to estimate calcium intake. Effect modification by calcium intake (dichotomized at 800 mg/day) was examined using interaction terms in logistic regression models and by conducting analyses stratified on the calcium variable. Increasing bone and blood Pb increased the odds of hypertension, particularly among subjects with low dietary calcium (i.e., <800 mg/day).

Zhang et al. (2010a) examined the effect of polymorphisms of the hemochromatosis gene (HFE) on the relationship of bone Pb with PP in NAS men. HFE polymorphisms promote Fe absorption and have been shown to modify the impact of adult cardiac function. The H63D variant is associated with hemochromatosis, a disease characterized by higher Fe body burden. Subjects had up to three PP measurements during the 10 year study period. The overall results demonstrated a strong relationship between bone Pb and PP in this study, similar to an earlier cross-sectional PP study of many of the same subjects (Perlstein et al., 2007). Zhang et al. (2010a) extended these findings by demonstrating

larger increases in PP per unit increase in tibia and patella Pb level among those with the H63D variant compared to those with the wild-type or the C282Y variant.

A small number of cross-sectional studies examined and found that blood Pb level was associated with hypertension in pregnancy. Yazbeck et al. (2009) examined a community-based group of pregnant women in France and unlike most other studies, adjusted for potential confounding by blood concentrations of Cd, Mn, and Se. Pregnancy induced hypertension (PIH) was defined as systolic BP >140 mmHg and/or diastolic BP >90 mmHg during at least two clinic visits after week 22 of gestation. Patients with pre-existing chronic hypertension were excluded. The mean (SD) blood Pb levels measured during pregnancy were 2.2 (1.4) µg/dL in PIH cases and 1.9 (1.2) µg/dL in normotensive women. An association between blood Pb and PIH was observed (OR 3.29 [95% CI: 1.11, 9.74] per unit increase in log-transformed blood Pb level). Cd and Se concentrations were comparable between PIH and no PIH groups. Adjustment for the metals slightly attenuated but did not eliminate the association between blood Pb levels and the risk of PIH. Investigators observed no significant interactions among blood Pb level, any of the other elements, and maternal characteristics in predicting the risk of PIH. Interaction between blood Se and Pb concentrations was not significant, and the putative protection effects of Se through antioxidative properties were not found in this study.

Wells et al. (2011b) measured the relationship of cord blood Pb with BP in 285 women at admission to the Johns Hopkins Hospital in Baltimore, MD, during labor and delivery. Women with cord blood Pb levels in the highest quartile for the study group (>0.96 µg/dL) had significantly higher systolic and diastolic BP (upon admission and for maximum BP) compared to women in the first quartile (<0.46 µg/dL). The level of uncertainty at these levels of exposure is difficult to estimate. The authors used Benchmark Dose Software V2.1, developed by the EPA, to estimate the blood Pb level (benchmark dose or BMD) and the associated lower confidence limit (BMDL) that was associated with one SD increase in BP. In this study group, one SD is approximately equivalent to a 10% increase above the mean for the first quartile blood Pb reference group. The BMD approach was used only as a means of quantifying the relationship of blood Pb with BP in this population. This analysis indicated that the BMDL for the maternal blood Pb level (estimated from cord blood Pb levels) that was associated with a 1 SD increase in all BP outcomes was about 1.4 µg/dL. These reported results are similar to those reported in the 2006 Pb AQCD as well as those found 25 years ago but with blood Pb levels an order of magnitude lower in the more recent study. However, uncertainty exists as to the specific Pb exposure level, timing, frequency, and duration that contributed to the observed associations.

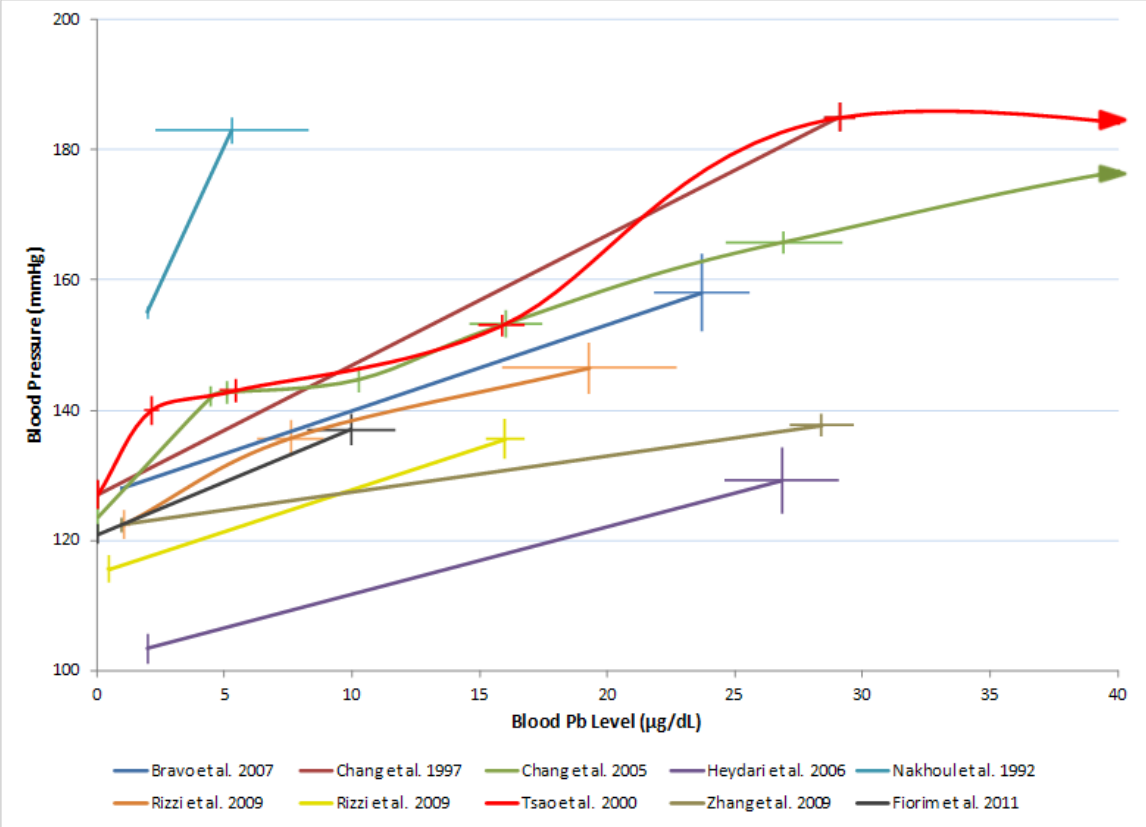
Recent analyses using NHANES data continued to indicate associations of Pb biomarkers with BP and hypertension. Muntner et al. (2005) previously used the NHANES 1999-2002 data to indicate that concurrent blood Pb levels were associated with hypertension, peripheral artery disease (PAD), and chronic kidney disease. The PAD results are discussed later in Section 4.4.3.5, and chronic kidney disease results are discussed in Section 4.5.2.1. Blood Pb increased regularly with age (geometric means [95% CIs]: 1.28 µg/dL [1.23, 1.33] in the 18-39 year age group to 2.32 µg/dL [2.20, 2.44] in the 75 year and older age group). Associations were observed between concurrent blood Pb level and hypertension across race/ethnicity groups with significant trends observed for non-Hispanic blacks and Mexican Americans.

In the NHANES III 1988-1994 population, Scinicariello et al. (2010) found a gene-environment interaction between blood Pb level and ALAD genotype (the genotypes have different affinities for Pb) in relation to systolic BP and diastolic BP in a cross-sectional analysis. These interactions varied across race/ethnicity strata. The strongest associations were observed among non-Hispanic blacks (Figure 4-17, Table 4-18). A statistically significant interaction was observed between concurrent blood Pb level and ALAD1-2/2-2b (i.e., ALAD2 carriers) among non-Hispanic whites and non-Hispanic blacks. Scinicariello et al. (2010) also found an interaction between ALAD genotype and blood Pb level in the association with hypertension. Statistically significant associations between concurrent blood Pb level and hypertension were observed among non-Hispanic blacks and nonsignificant increases were observed among non-Hispanic whites and Mexican Americans (with the exception of Mexican Americans in the second quartile of blood Pb level) (Figure 4-18, Table 4-19). In addition, non-Hispanic white ALAD2 carriers in the highest blood Pb level quartile 3.8-52.9 µg/dL) had a significantly higher association with hypertension compared with ALAD1 homozygous individuals in the highest quartile of blood Pb. In the same NHANES population, Park et al. (2009b) predicted bone Pb levels using a model developed with NAS data. Concurrent blood Pb was associated with hypertension overall in the NHANES population, with larger associations observed among black men and women as well as older adults (Figure 4-18, Table 4-19). Associations also were observed with estimated bone Pb.

4.4.2.2 Toxicology

Studies on the effect of Pb (as blood Pb level) on systolic BP in unanesthetized adult rats consistently reported an increase in BP with increasing blood Pb level as shown in Figure 4-20 (results summarized in Table 4-20). An array of studies has provided evidence that long-term Pb exposure (>4 weeks), resulting in blood Pb levels less than 10 µg/dL, which is relevant to humans in the U.S., can result in the onset of hypertension (after a latency

period) in experimental animals that persists long after the cessation of Pb exposure (U.S. EPA, 2006b). Tsao et al. (2000) presented evidence for increased systolic and diastolic BP in rats with blood Pb levels somewhat similar to the current U.S. adult population (mean [SD]: 2.15 [0.92] µg/dL blood Pb; 140 [7] mmHg systolic BP, 98 [7] mmHg diastolic BP) compared to untreated controls (mean [SD]: 0.05 [0.05] µg/dL blood Pb; 127 [7] mmHg systolic BP, 88 [7] mmHg diastolic BP). As this was the lowest Pb level tested, no evidence of a threshold was evident. Further, a test for linear trend revealed a statistically significant, positive trend for increasing BP with increasing blood Pb levels up to 56 µg/dL (e.g., mean [SD]: 5.47 [2.1] µg/dL blood Pb; 143 [6] mmHg systolic BP, 97 [8] mmHg diastolic BP), with the effect leveling off at higher blood Pb levels.



Note: Crosses represent standard error for blood Pb and BP measurements. If no crossbar is present, error results were not reported. Arrows represent higher doses tested.

Figure 4-20 Changes in BP after Pb exposure (represented as blood Pb level) in unanesthetized adult rats across studies.

Table 4-20 Characteristics of studies of blood Pb with BP measures in animals presented in Figure 4-20.

Reference[a]	Lifestage; Sex; Strain	Exposure Duration	Exposure Level; Route	Mean [SEM][b] Blood Pb Level (µg/dL)	n	ΔSBP (mmHg; lowest blood Pb level compared with control)[c]	Comments
Fiorim et al. (2011)	Adult; M, Wistar Rat	7 days	4 µg/100 g followed by 0.05 µg/100 g daily; intramuscular	9.98 [1.7]	12	16	
Nakhoul et al. (1992)	Adult; M SH Rat	8 weeks	100 ppm; drinking water	5.3 [3]	7	28	Spontaneously hypertensive rat model
Chang et al. (2005)	Adult; M Wistar Rat	8 weeks	20,000 ppm then removal and measurements 1-7 mo after; drinking water	Range: 4.5 to 83	5	13.8	
Tsao et al. (2000)	Adult, M Wistar Rat	8 weeks	100 - 20,000 ppm; drinking water	Range of means: 2.15 [0.29] to 85.76 [1.29][b]	10	13	
Rizzi et al. (2009)	Adult; M Wistar Rat	8 weeks	30, 90 ppm; drinking water	7.6 [1.3], 19.3 [3.4]	11	13.3	
Chang et al. (1997)	Adult; M Wistar Rat	8 weeks	500 ppm; drinking water	29.1 [0.6][b]	10	58	
Heydari et al. (2006)	Adult; M Sprague-Dawley Rat	12 weeks	100 ppm; drinking water	26.8 [2.2]	6	25.8	
Bravo et al. (2007)	Adult; M Sprague-Dawley Rat	14 weeks	100 ppm; drinking water	23.7 [1.9][b]	12	30	
Zhang et al. (2009a)	Adult; M Wistar Rat	40 weeks	100 ppm; drinking water	28.4 [1.1][b]	8-10	15.3	

[a]Studies are presented in order of increasing duration of exposure.

[b]Standard deviation converted to SEM.

[c]Difference in systolic BP (SBP) between group means not within one exposure group.

Experimental animal studies continued to provide evidence that long-term Pb exposure results in sustained arterial hypertension after a latency period. Systolic BP increased in rats after exposure to 90-10,000 ppm Pb (as Pb acetate in drinking water) for various time periods that resulted in blood Pb levels between 19.3-240 µg/dL (Mohammad et al., 2010; Zhang et al., 2009a; Badavi et al., 2008; Grizzo and Cordellini, 2008; Reza et al., 2008; Bravo et al., 2007; Vargas-Robles et al., 2007; Heydari et al., 2006; Bagchi and Preuss, 2005). Past studies have shown statistically significant elevations in BP in rats with lower blood Pb levels. For example, long-term Pb exposure to spontaneously hypertensive rats (resulting in mean [SEM] blood Pb level: 5.3 [3] µg/dL) led to increased BP (Nakhoul et al., 1992). Consistent with measurements of systolic BP by tail-cuff plethysmography, Pb exposure (100 ppm for 14 weeks; mean blood Pb level: 24 µg/dL) also caused an increase in intra-aortic mean arterial pressure (Bravo et al., 2007). In a study that tested low levels of Pb exposure (30 ppm; mean blood Pb level: 7.6 µg/dL), a statistically significant increase in systolic BP was not observed despite elevated blood Pb level after 8 weeks of treatment. Nonetheless, there was a trend of higher BP with increased blood Pb levels (Rizzi et al., 2009).

Studies found that Pb-induced increases in BP persisted long after cessation of Pb exposure. Bagchi and Preuss (2005) found that elevated systolic BP was maintained for 210 days after cessation of Pb exposure (10,000 ppm Pb acetate in water, 40 days, monitored for one year). However, chelation therapy using $Na_2CaEDTA$ returned systolic BP to levels comparable to those in rats not treated with Pb (Bagchi and Preuss, 2005). Chang et al. (2005) reported a partial reversibility of effect after cessation of Pb exposure, where Pb-induced elevated BP decreased but did not return to control levels 7 months post Pb exposure. After Pb exposure was removed, blood, heart, aorta, and kidney Pb levels decreased quickly within the first three months (Chang et al., 2005). Pb-induced elevated systolic BP persisted for one month following Pb exposure cessation, followed by obvious decreases in BP until 4 months after Pb exposure cessation. Between 4 and 7 months after Pb exposure cessation, the still-elevated BP did not decrease further, thus never returning to control BP levels. Decreases in BP were strongly correlated with decreases in blood Pb level after exposure cessation.

The aforementioned studies all assessed the relationship between long-term exposure (>4 weeks) of rats to Pb and measures of BP. However, recent research also investigated BP elevation occurring after short-term treatment with Pb (<4 weeks). Studies found increased systolic BP after 7 days of Pb treatment (daily injections resulting in mean [SEM] blood Pb levels of 9.98 [1.7] µg/dL) (Fiorim et al., 2011) and after 2 weeks of Pb exposure (100 ppm via drinking water) (Sharifi et al., 2004). A study utilizing intra-arterial pressure measurements found that a single high-dose Pb injection in rats (resulting in mean [SEM] blood Pb levels of 37 [1.7] µg/dL) increased systolic arterial

pressure after only 60 minutes (Simões et al., 2011). The injection of Pb into the rat may not allow for extrapolation of these results to humans since this is not a comparable Pb exposure route. These studies suggest that there is the potential for an increase in BP following short-term Pb treatment. It is possible that the increases in BP following short- and long-term Pb exposures are occurring through separate mechanisms; however, studies using both short- and longer-term Pb exposure have correlated increased BP with an activation of the renin-angiotensin system (i.e., increase in angiotensin converting enzyme (ACE) activity) (Section 4.4.2.3). Several of these aforementioned studies used the injection route of Pb administration, and the relevance of these bolus doses over short periods of time to human routes of short-term exposure is uncertain. However, it is important to acknowledge that the results were similar to those from the study that examined short-term exposure to Pb via drinking water.

4.4.2.3 Hypertension Modes of Action

The 2006 Pb AQCD (U.S. EPA, 2006b) examined a number of mechanisms leading to Pb-induced hypertension, including oxidative stress, hormonal and blood pressure regulatory system dysfunction, vasomodulation, and cellular alterations. As described below, recent studies in experimental animals and cells further supported roles for these potential mechanisms in mediating hypertension from Pb exposure.

Oxidative Stress Response - Reactive Oxygen Species and Nitric Oxide

Several studies discussed in the 2006 Pb AQCD demonstrated a role for oxidative stress in the pathogenesis of Pb-induced hypertension, mediated by the inactivation of nitric oxide (NO) and downregulation of soluble guanylate cyclase (sGC) (Dursun et al., 2005; Attri et al., 2003; Gonick et al., 1997; Vaziri et al., 1997; Khalil-Manesh et al., 1994; Khalil-Manesh et al., 1993b). Pb-induced reduction of biologically active NO was found not to be due to a reduction in NO-production capacity (Vaziri and Ding, 2001; Vaziri et al., 1999a); instead, it was found to result from inactivation and sequestration of NO by ROS (Malvezzi et al., 2001; Vaziri et al., 1999b). Oxidative stress from Pb exposure in animals may be due to upregulation of NAD(P)H oxidase (Ni et al., 2004; Vaziri et al., 2003), induction of Fenton and Haber-Weiss reactions (Ding et al., 2001; Ding et al., 2000), and failure of the antioxidant enzymes, CAT and GPx, to compensate for the increased ROS (Farmand et al., 2005; Vaziri et al., 2003). Many biological actions of NO, such as vasorelaxation, are mediated by cGMP, which is produced by sGC from the substrate GTP. Oxidative stress also has been found to play a role in Pb-induced downregulation of sGC (Farmand et al., 2005; Courtois et al., 2003; Marques et al.,

2001). Thus, the reduction of the vasodilator NO from inactivation and sequestration by Pb-induced ROS leads to increased vasoconstriction and BP.

Pb-induced oxidative stress also has been found to induce renal tubulointerstitial inflammation which plays a crucial role in models of hypertension (Rodriguez-Iturbe et al., 2005; Rodriguez-Iturbe et al., 2004). Tubulointerstitial inflammation from treatment with Pb has been coupled with activation of the redox sensitive NF-κB (Ramesh et al., 2001). Pb-induced hypertension, inflammation, and NF-κB activation can be ameliorated by antioxidant therapy (Rodriguez-Iturbe et al., 2004). There is mixed evidence to suggest that Pb-induced hypertension may also be promoted by activation of PKC leading to enhanced vascular contractility (Valencia et al., 2001; Watts et al., 1995).

Recent studies continued to provide evidence for the role of ROS and NO metabolism in Pb-induced hypertension and vascular disease. Increased systolic BP after Pb exposure was accompanied by increased superoxide (O_2^-) and O_2^--positive cells (Bravo et al., 2007; Vargas-Robles et al., 2007), elevated urinary malondialdehyde (MDA, a measure of lipid peroxidation) (Bravo et al., 2007), and increased 3-nitrotyrosine (Vargas-Robles et al., 2007). Inhibition of NAD(P)H oxidase, an enzyme that generates O_2^- and hydrogen peroxide, was able to block Pb-induced (1 ppm) aortic contraction to 5-hydroxytryptamine (5-HT) (Zhang et al., 2005). Increases in systolic BP, intra-aortic mean arterial pressure, and MDA after Pb exposure (100 ppm; mean blood Pb level: 23.7 µg/dL) were also prevented by treatment with the immunosuppressant, mycophenolate mofetil (MMF) (mean blood Pb level in MMF-treated animals: 27 µg/dL) (Bravo et al., 2007). MMF has been shown to inhibit endothelial NAD(P)H oxidase, which could explain how it decreases Pb-induced increases in oxidative stress and BP. MMF was not found to alter blood Pb levels of animals. Red grape seed extract and ascorbic acid supplementation were also able to protect rats from Pb-induced (100 ppm) increased BP and heart rate, perhaps through the antioxidant properties of both the extract (Badavi et al., 2008) and vitamin C (Mohammad et al., 2010). Red grape seed extract did not alter the accumulation of Pb in blood, indicating that its protective effect was not mediated through altered Pb toxicokinetics; however, internal doses of Pb were not measured in the vitamin C study to clarify the mechanism of action of vitamin C. Another study found that the antioxidant, anti-inflammatory chemical, curcumin, as well as physical exercise training reversed Pb-induced increases in serum creatinine kinase-MB (CK-MB), low density lipoprotein (LDL), heart high-sensitivity C-reactive protein (hs-CRP), and MDA. Pb-induced decreases in serum total antioxidant capacity, high density lipoprotein (HDL), and heart glutathione peroxidase (GPx) were also reversed by curcumin and exercise. However, internal doses of Pb were not measured to clarify the mechanism of action in this study (Roshan et al., 2011).

Exposure to Pb can also affect the activity and levels of antioxidant enzymes. Male (♂) and female (♀) rats exposed to Pb for 18 weeks (100-1,000 ppm) had altered responses in antioxidant enzymes in heart tissue (Sobekova et al., 2009; Alghazal et al., 2008a). Pb exposure in female rats increased the activity of cardiac SOD, GST, GR, and GPx (>100 ppm) and increased cardiac thiobarbituric acid reactive substances (TBARS, a measure of lipid peroxidation) (1,000 ppm). Pb exposure in male rats did not affect the activity of SOD or production of TBARS, however decreased the activity of GST and GR (>100 ppm). Male and female rats also accumulated different amounts of Pb in the cardiac tissue after similar Pb exposure (♂ 100 ppm: 205% of control, 1,000 ppm: 379%; ♀ 100 ppm: 246%, 1,000 ppm: 775%), which could explain the sex differences observed in antioxidant enzyme responses.

Oxidative stress can trigger a cascade of events that promote cellular stress, renal inflammation, and hypertension. As was shown previously (Rodriguez-Iturbe et al., 2005), Pb exposure can increase renal NF-κB, which was associated with tubulointerstitial damage and infiltration of lymphocytes and macrophages (Bravo et al., 2007). These events could also be ablated by MMF treatment, likely due to its anti-inflammatory and antioxidant properties. Pb also was found to induce inflammation in human endothelial cells as a model for vessel intima hyperplasia (Zeller et al., 2010). The pro-inflammatory cytokine, interleukin (IL)-8 protein and mRNA were increased, concentration- and time-dependently, after in vitro Pb exposure (5-50 µM). Enhanced IL-8 production was mediated through activation of the transcription factor Nrf2 (but not NF-κB, hypoxia inducible factor-1, or aryl hydrocarbon receptor), as shown through increased nuclear translocation and Nrf2 cellular knockdown experiments. Additionally, measures of endothelial stress, NQO1 and HO-1 protein, were induced by Pb exposure (Zeller et al., 2010). Pb treatment (20 ppm, i.p., 3 days/week, 8 weeks) increased the inflammatory markers hs-CRP and CK-MB in rat hearts (Roshan et al., 2011).

Oxidative stress affects vascular reactivity and tone through inactivation and sequestration of NO, causing a reduction in biologically active NO. Recent studies affirmed past conclusions on the interplay of ROS and NO metabolism in the cardiovascular effects of Pb. Elevated systolic BP and altered vasorelaxation after Pb exposure was accompanied by a decrease in total nitrates and nitrites (NO_X) (Mohammad et al., 2010; Zhang et al., 2007a; Heydari et al., 2006). Serum NO_X levels in Pb-treated rats remained depressed for 8 weeks and then reversed after 12 weeks, despite continued elevation in systolic BP (Heydari et al., 2006). This return of serum NO_X levels to levels similar in controls could be a result of compensatory increases in endothelial NOS (eNOS) attempting to replenish an over-sequestered NO supply. With this in mind, studies showed increased eNOS protein expression after long-term Pb exposure in kidney (Zhang et al., 2007a) and isolated cultured aorta (Vargas-Robles et al., 2007). No change

in inducible NOS was observed in isolated cultured aorta after 1 ppm Pb exposure (Zhang et al., 2007a). In contrast to long-term exposure, Pb treatment over a short time period (daily injections resulting in mean [SEM] blood Pb levels of 9.98 [1.7] µg/dL) was found to increase iNOS and phosphorylated eNOS protein (Fiorim et al., 2011) which may cause an increase in NO production and a short-term increase in NO bioavailability. This increase in NO bioavailability early after Pb exposure could be the immediate compensatory mechanism against the elevation in BP.

NO, also known as endothelium-derived relaxing factor, is a potent endogenous vasodilator. Toxicological studies continued to investigate the effects of Pb on NO-dependent vascular reactivity by using NO stimulating vasodilators, such as acetylcholine (ACh) and sodium nitroprusside (SNP), and NO inhibiting vasoconstrictors, such as L-NAME. Studies provided mixed evidence; however, results suggested that Pb disrupts the vasorelaxant response to NO in the aorta due to damage to the endothelium. Pb exposure (1 ppm and 100 µM, 1 hour) decreased ACh-induced vasorelaxation, which triggers the release of NO from the endothelial cell, in isolated rat tail artery, suggesting damage to the endothelium (Silveira et al., 2010; Zhang et al., 2007a). In aortic rings of perinatally exposed rats (1,000 ppm through pregnancy and lactation, mean blood Pb level: 58.7 µg/dL), blocking NOS with L-NAME abolished the relaxant response evoked by ACh (Grizzo and Cordellini, 2008). However, there was no change observed in the relaxation response to ACh by Pb alone (Fiorim et al., 2011; Rizzi et al., 2009; Grizzo and Cordellini, 2008). Conversely, Skoczynska and Stojek (2005) found that Pb exposure (50 ppm; blood Pb level 11.2 µg/dL) enhanced NO-mediated vasodilation by ACh in rat mesenteric arteries, and NOS inhibition enhanced the ACh relaxant response. A number of studies found that Pb exposure did not affect smooth muscle integrity since SNP-induced vasorelaxation, which is endothelium independent, was unchanged (Fiorim et al., 2011; Silveira et al., 2010; Rizzi et al., 2009; Grizzo and Cordellini, 2008).

NO also was found to play a role in the interaction between Pb and the vasoconstrictor response. Blocking NOS with L-NAME or inhibiting iNOS specifically, which decreases NO production, increased the contraction of aortic rings in response to the vasoconstrictor phenylephrine (PHE), and Pb exposure potentiated this response (Fiorim et al., 2011). Also, L-NAME increased the Pb pressor response to PHE after perinatal Pb exposure (1,000 ppm through pregnancy and lactation, blood Pb level 58.7 µg/dL) (Grizzo and Cordellini, 2008). Conversely, in rat renal interlobar arteries, Pb exposure blunted the increase in renal angiotensin II (AngII)-mediated contraction from NOS inhibition by L-NAME (Vargas-Robles et al., 2007). Treatment with the SOD mimetic tempol, which would increase NO bioavailability, decreased, but did not eliminate, the Pb pressor response (Silveira et al., 2010).

In summary, recent studies continued to provide evidence for the role of ROS in Pb-induced hypertension and vascular disease by indicating Pb-induced increases in ROS and modulation of cardiovascular responses by antioxidant substances. Additionally, recent studies continued to show that Pb-induced hypertension and vascular responses are mediated primarily via inactivation of NO not via inhibition of NO production.

Vascular Reactivity

Pb exposure-induced alterations of the adrenergic system, which can increase peripheral vascular resistance, and thereby arterial pressure, may be one mediator of Pb-induced hypertension. Pb exposure in animals can increase stimulation of the sympathetic nervous system (SNS), as shown by increased plasma levels of norepinephrine (NE) and other catecholamines (Carmignani et al., 2000; Chang et al., 1997) and decreased β adrenergic receptor density and β agonist-stimulated cAMP production in the aorta and heart (Tsao et al., 2000; Chang et al., 1997). These stimulatory effects on the SNS paralleled the effects of Pb on BP, cardiac contractility, and carotid blood flow. Pb-induced elevations in arterial pressure and heart rate were abrogated by ganglionic blockade (Simões et al., 2011; Lai et al., 2002). Arterial pressure and heart rate gradually decreased 7 months after Pb exposure cessation as did the Pb-induced SNS alterations (Chang et al., 2005).

Increases in BP can be caused by activation of the SNS, which can lead to vascular narrowing, in turn, resulting in increased total peripheral resistance. In this neural mechanism, activation of the SNS leads to vasoconstriction, whereas inhibition leads to vasodilation. It has been suggested that Pb leads to increased vascular reactivity to catecholamines (i.e., epinephrine, NE, and dopamine), hormones of the SNS. Indeed, the isolated mesenteric vessel bed from Pb-treated rats (50 ppm with blood Pb level: 11.2 µg/dL, but not 100 ppm with blood Pb level: 17.3 µg/dL) exhibited increased reactivity to NE (Skoczynska and Stojek, 2005). However, in another study, 100 ppm Pb did not affect the NE-induced contractile response after 10 months of exposure (Zhang et al., 2009a), suggesting a small range of Pb doses affects pressor response to NE. Catecholamines act primarily through the adrenergic and dopaminergic receptors. Antagonists of α1-adrenergic, α2-adrenergic, β-adrenergic, and dopamine D1 receptors were found to abolish Pb-induced aortic contraction (Fazli-Tabaei et al., 2006; Heydari et al., 2006). However, the α1-adrenergic receptor agonist, PHE, induced aortic contractions and these were enhanced by treatment with Pb (100 ppm; blood Pb level: 26.8 µg/dL), indicating a specific role for the α1-adrenergic receptor (Silveira et al., 2010; Grizzo and Cordellini, 2008; Heydari et al., 2006). Removal of the endothelium blunted the PHE-induced contraction. Conversely, short-term Pb treatment (7 days, i.p.) decreased the contractile response induced by PHE in rat aortas resulting in a decreased vascular reactivity (Fiorim et al., 2011). This decrease may represent a compensatory response

attempting to correct the Pb-induced BP elevation. Additionally, Pb blunted the isoproterenol-induced relaxation, supporting a role for the β-adrenoceptors in mediating the effects of Pb on vascular reactivity (Vassallo et al., 2008; Heydari et al., 2006).

Recently, there was mixed evidence for Pb disrupting vascular reactivity to other pressor agents. Pb (1 ppm) treatment of isolated rat thoracic aorta increased 5-HT induced contraction, which was endothelium dependent, but not due to 5-HT2B receptor expression (Zhang et al., 2005). Follow-up of this study in whole animals found, on the contrary, that Pb (100 ppm; blood Pb level: 28.4 µg/dL) decreased the maximum contractile response to 5-HT, but did not affect 5-HT plasma levels or 5-HT2B receptor expression (Zhang et al., 2009a). In addition, Pb exposure (100 ppm, 12 weeks) increased the renal vascular response to AngII in isolated perfused kidneys from Pb-exposed rats (Vargas-Robles et al., 2007).

Studies continued to investigate the effects of Pb on NO-dependent vascular reactivity by using NO stimulating vasodilators, such as ACh and SNP, and NO inhibiting vasoconstrictors, such as L-NAME. These studies were discussed in a preceding subsection (Oxidative Stress Response).

Renin-Angiotensin-Aldosterone and Kininergic Systems

The adrenergic system also affects the renin-angiotensin-aldosterone system (RAAS), which is responsible for fluid homeostasis and BP regulation, and has been shown to be affected by Pb exposure. A meta-analysis found that Pb exposure (resulting in blood Pb levels: 30-40 µg/dL) increased plasma renin activity and renal tissue renin in young but not old rats (Vander, 1988). Exposure of experimental animals to Pb also induced increases in plasma, aorta, heart, and kidney angiotensin converting enzyme (ACE) activity; plasma kininase II, kininase I, and kallikrein activities; and renal AngII positive cells (Rodriguez-Iturbe et al., 2005; Sharifi et al., 2004; Carmignani et al., 1999). ACE activity declined over time while arterial pressure stayed elevated, suggesting that the RAAS may be involved in the induction, but not the maintenance of Pb-induced hypertension in rats.

Recent studies continued to implicate the RAAS in the development of Pb-induced hypertension, especially during early exposure in young animals. AngII, a main player in the RAAS, induces arteriolar vasoconstriction leading to increased BP. Pb exposure increased the vascular reactivity to AngII (Vargas-Robles et al., 2007). Acute (60 minutes) or short-term (7 days) treatment of rats to Pb increased the plasma ACE activity (Fiorim et al., 2011; Simões et al., 2011), and Fiorim et al. (2011) additionally found this increase to be correlated with the Pb-induced increase in systolic BP.

However, after these short time points there were no changes in the AngII receptors 1 or 2 protein levels or expression. Treatment with the AngII receptor (AT_1R) blocker, Losartan, or the ACE inhibitor, Enalapril, blocked the Pb-induced systolic BP increase (Simões et al., 2011) and decreased the PHE-induced vasoconstrictor response in Pb-treated aortas (Fiorim et al., 2011). Similarly, treatment with Losartan resulted in a greater decrease in systolic BP in highly Pb-exposed rats (10,000 ppm Pb, 40 days; blood Pb level >240 µg/dL after exposure, 12-13 µg/dL after chelation after 1 year) compared to control rats, and this decrease continued into later periods of follow-up (day 283) (Bagchi and Preuss, 2005). Increased systolic BP after early exposure to Pb corresponded with increased water intake, urine output, potassium excretion, and decreased urinary sodium and urine osmolality. These functional changes in renal behavior are consistent with the actions of a stimulated RAAS. Lower level Pb (100 ppm, 14 weeks; range of blood Pb levels: 23.7-27 µg/dL) exposure increased renal cortical AngII content and the number of tubulointerstitial AngII-positive cells (Bravo et al., 2007). This heightened intrarenal angiotensin corresponded with sodium retention and increased systolic BP and was ablated by the anti-inflammatory antioxidant, MMF. Sodium reabsorption is important for the maintenance of BP, and Na^+ transporters play a key role in this process. In other studies, Pb exposure increased activity and levels of the α-1 subunit protein of Na^+/K^+ATPase, which plays a major role in Na^+ reabsorption and is regulated by the RAAS (Fiorim et al., 2011; Simões et al., 2011). These studies point to the activation of the RAAS in the course of Pb-induced hypertension, particularly in the early stages of elevated BP.

Vasomodulators

The balance between production of vasodilators and vasoconstrictors is important in the regulation of BP and cardiovascular function. The 2006 Pb AQCD reported that Pb did not affect all vasomodulators in the same way. Urinary excretion of the vasoconstrictor, thromboxane (TXB_2), and the vasodilatory prostaglandin, 6-keto-PGF1α, was unchanged in rats with Pb-induced hypertension (Gonick et al., 1998). However, in vitro Pb exposure promoted the release of the prostaglandin precursor, arachidonic acid, in vascular smooth muscle cells (VSMCs) via activation of phospholipase A2 (Dorman and Freeman, 2002). Plasma concentration and urinary excretion of the vasoconstrictive peptide, endothelin (ET)-3 was increased after low- (100 ppm), but not high-level (5,000 ppm) Pb exposure in rats (Gonick et al., 1997; Khalil-Manesh et al., 1994; Khalil-Manesh et al., 1993b). Antagonism of the ET receptor A blunted the downregulation of sGC and cGMP production by Pb in isolated rat artery segments, suggesting that some of the hypertensive effects of Pb exposure may be mediated through ET (Courtois et al., 2003). Additionally, Pb-exposed animals exhibited fluid retention and a

concentration-dependent decline in the vasodilator, atrial natriuretic factor (ANF) (Giridhar and Isom, 1990). Results from these studies suggest that Pb may interfere with the balance between vasodilators and vasoconstrictors that contribute to the complex hormonal regulation of vascular contraction and BP.

The imbalance in vasomodulators is one explanation for the concentration-dependent vasoconstriction observed in some animals after Pb exposure (Valencia et al., 2001; Watts et al., 1995; Piccinini et al., 1977). However, vasoconstriction after Pb exposure was not reported in all studies (Shelkovnikov and Gonick, 2001) and is likely varied depending on the type of vessel used, the Pb concentration employed, and the animal species being studied. Studies have reported Pb-induced attenuation of ACh-mediated and NO-mediated vasodilation (Marques et al., 2001; Oishi et al., 1996) in some, but not all vascular tissues and in some, but not all studies (Purdy et al., 1997). These effects have been variably attributed to Pb-mediated activation of PKC and direct action on the VSMCs through the Ca^{2+} mimetic properties of Pb among other possibilities (Valencia et al., 2001; Watts et al., 1995; Piccinini et al., 1977).

A recent study investigated the role of the endothelial-derived vasoconstrictor, ET-1, in Pb-induced hypertension. ET-1 from the endothelium acts on the ET_A-type receptors located on the vascular smooth muscle layer and may be involved in vascular reactivity by NO and cyclooxygenase (COX) derivatives. Pb exposure (1 ppm, 24 hours) to rat aortic segments decreased expression of sGC-β1 subunit, an enzyme involved in NO-induced vasodilation, and increased expression of COX-2 in an endothelium-dependent manner (Molero et al., 2006). Even though Pb treatment did not alter ET-1 or ET_A-type receptor protein expression in this system, blocking the ET_A-type receptors partially reversed Pb-induced changes in sGC and COX-2 in vascular tissue. These results suggest that the endothelium and ET-1 may contribute to Pb-induced hypertension through activation of ET_A-type receptors that alter expression of COX-2 and sGC-β1 subunit, which affects NO signaling.

COX-2 blockade has been shown to prevent Pb-induced downregulation of sGC expression (Courtois et al., 2003). Inhibition of COX-2 also decreased the Pb-induced pressor response to ACh (Grizzo and Cordellini, 2008) and PHE (Silveira et al., 2010) in experimental animals. These results suggest that Pb-induced vascular reactivity may depend on the participation of a COX-derived vasoconstrictor, such as prostaglandins, prostacyclins, or thromboxanes.

In summary, recent studies continued to show that Pb exposure affects vasomodulatory pathways that are important for the maintenance of vascular tone; however, results indicated that not all vascular cell types are similarly affected by Pb exposure. Further, effects appeared to vary according to the concentration of Pb exposure. Pb exposure has

been shown to interrupt baseline or endogenous NO-mediated vasodilation of vessels via alterations in PKC, sGC, VSMC, endothelial cells, NADPH oxidase, and Ca^{2+} levels. Recent studies indicated that Pb exposure may affect vascular reactivity by increasing COX-2 and COX-2-dependent vasoconstrictors. Also, the vasoconstrictor endothelin may contribute to Pb-induced vasomodulation via similar pathways as NO including effects on sGC and COX-2.

4.4.2.4 Summary of Blood Pressure and Hypertension

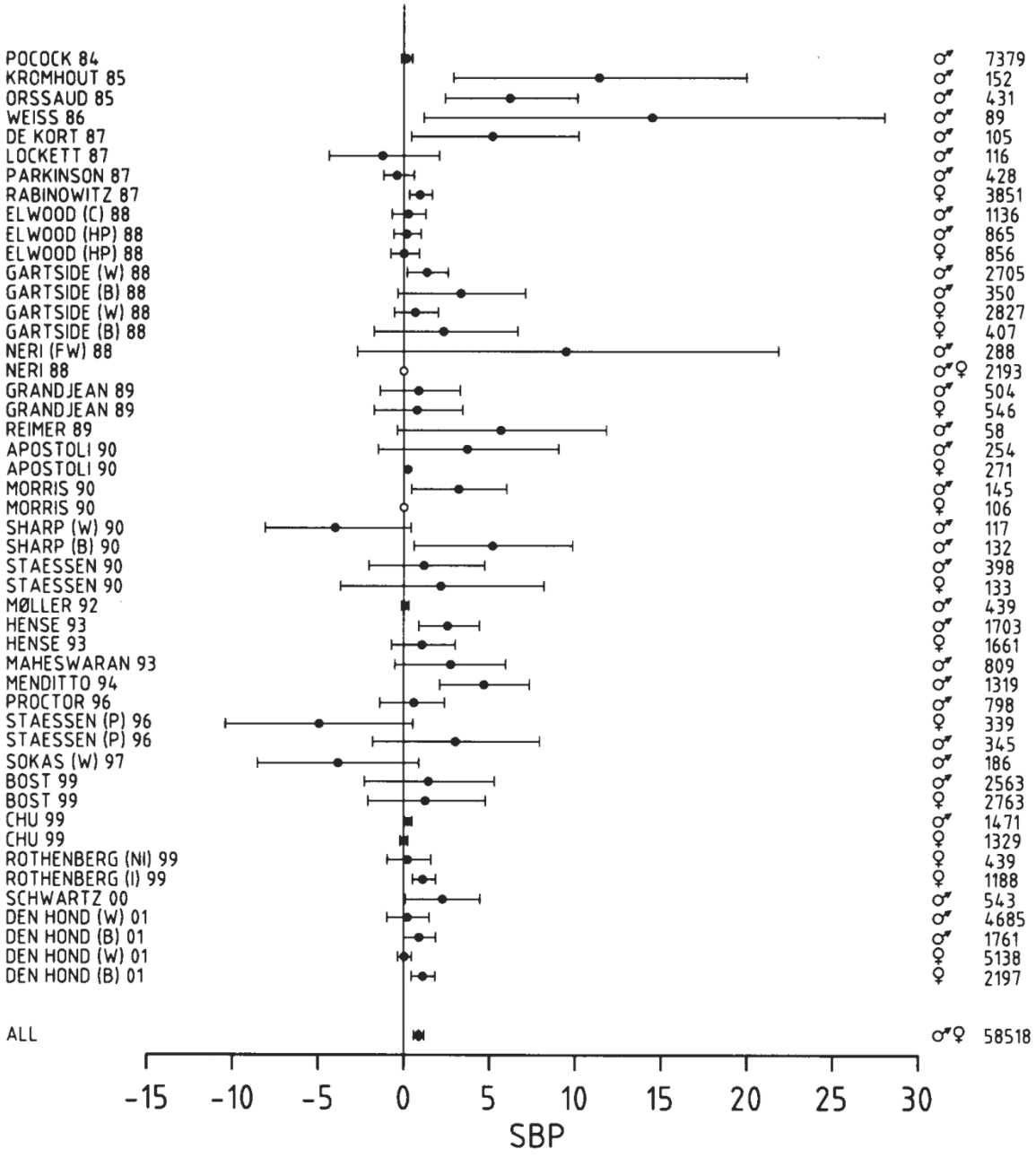
The 2006 Pb AQCD (U.S. EPA, 2006b) reported a clear association between higher blood Pb levels and higher BP. The effect was modest, but robust, as determined by a meta-analysis (Nawrot et al., 2002) of over 30 cross-sectional and prospective studies comprising over 58,000 adults (Figure 4-21). In the meta-analysis, each doubling of concurrent blood Pb was associated with a 1 mmHg increase in systolic BP and a 0.6 mmHg increase in diastolic BP. In addition, U.S. EPA (1990a) reviewed studies available prior to 1990, a period in the U.S. when Pb exposures from air were probably at the highest level, that examined Pb exposure and BP outcomes, which included several studies of the population represented in NHANES II (1976-80). They noted that across a blood Pb range of 7 to 34 µg/dL, no evident threshold was found below which the blood Pb level was not significantly related to BP. U.S. EPA (1990a) concluded that a small but positive association exists between blood Pb levels and increases in BP.

Recent epidemiologic studies supported this association at lower concurrent blood Pb levels (in populations with mean blood Pb levels < 2 µg/dL) and added to the evidence base regarding populations potentially at increased risk (i.e., high stress, genetic variants) and regarding associations of bone Pb levels with BP and hypertension in populations with mean bone Pb levels less than 20 µg/g. As these studies were mostly cross-sectional in design and were conducted in adults whose concurrent blood Pb levels are influenced both by current Pb exposures and past Pb exposures mobilized from bone, uncertainty exists over the Pb exposure conditions that contributed to the associations observed between concurrent blood Pb level and increased BP and hypertension (Sections 3.3 and 3.7.3).

A recent prospective study in Pb workers found independent associations of both baseline blood Pb level and subsequent changes in blood Pb over follow-up with changes in BP over follow-up and associations of bone Pb level with hypertension (Glenn et al., 2006). Although these Pb workers had higher current Pb exposure compared with nonoccupationally-exposed adults, the results indicated that different mechanisms may

mediate shorter-term Pb-associated increases in BP and longer-term Pb-associated development of hypertension.

Key evidence was further provided by a recent cross-sectional study in an ethnically diverse community-based cohort of women and men aged 50-70 years that reported associations between both blood and tibia Pb levels and BP with extensive consideration of potential confounding factors (Table 4-18) (Martin et al., 2006). Additionally, a recent epidemiologic study provided evidence for associations in an adult cohort between blood Pb level and BP and hypertension with relatively low blood Pb levels; a positive relationship was found in the NHANES adult data (1999-2002) with a geometric mean concurrent blood Pb level of 1.64 μg/dL (Muntner et al., 2005). However, as noted above, in adults, uncertainty exists regarding the magnitude, timing, frequency, and duration of Pb exposure that contribute to the associations observed with concurrent blood Pb levels.



Study Key: C - Caerphilly Study; HP - Welsh Heart Program; W - Whites; B - Blacks; NI - Non-immigrants; I - Immigrants; FW - Foundry Workers; CS - Civil Servants; P - PheeCad (Public Health and Environmental Exposure to Cadmium) Study.

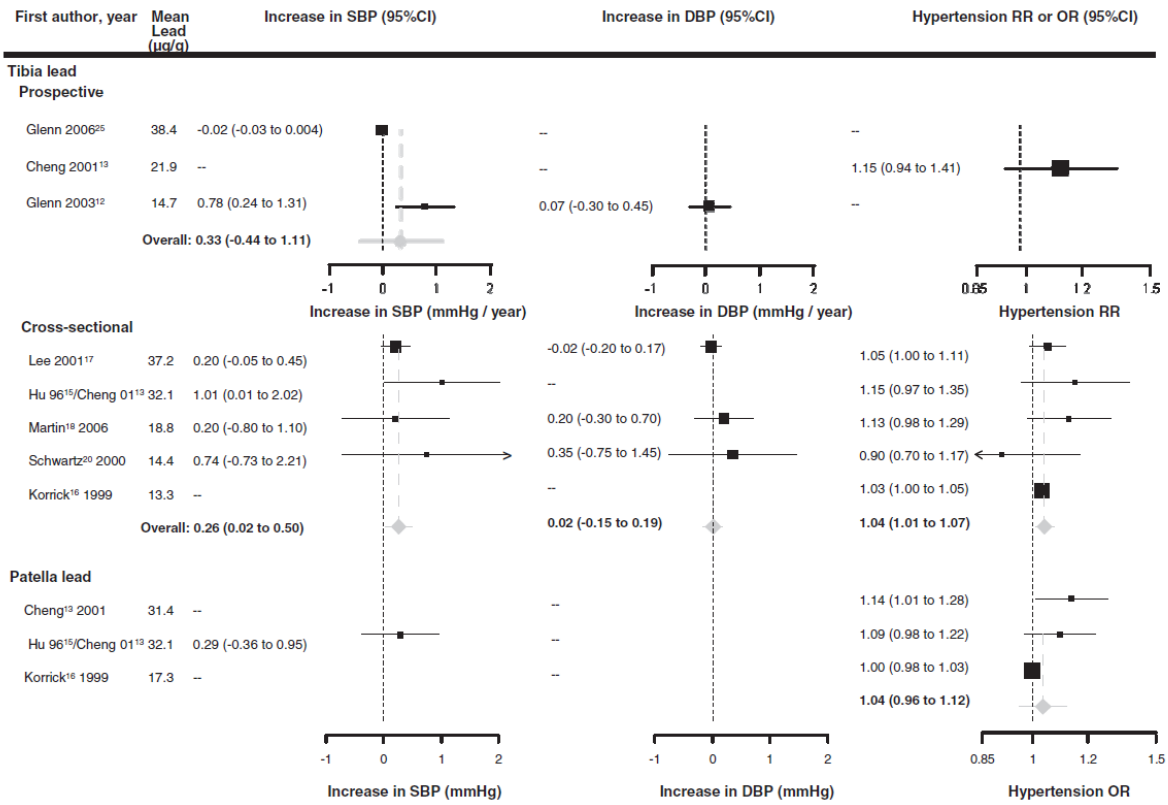
Note: Individual study results are presented in each row. The rightmost columns indicate the sex of subjects and study sample size. Circles represent individual groups and squares represent the combined association sizes. Open circles denote a nonsignificant association size that was assumed to be zero.

Source: Reprinted with permission of MacMillan Press, Nawrot et al. (2002)

Figure 4-21 Meta-analysis of change in systolic BP (SBP), in mmHg, with 95% CI, associated with a doubling in the blood Pb concentration.

In concordance with epidemiologic evidence, collectively, the animal toxicological studies providing blood Pb level and BP measurements reported higher BP with higher blood Pb levels in adult rodents (Figure 4-20). While the contribution of low concurrent blood Pb levels to the findings is difficult to ascertain in adult humans, animal toxicological studies provide support for low blood Pb level effects with increases in BP observed in groups of animals with long-term dietary Pb exposure resulting in blood Pb levels as low as 2 µg/dL (Rizzi et al., 2009; Tsao et al., 2000; Nakhoul et al., 1992). However, the majority of animal toxicological studies showing Pb-induced hypertension were conducted at higher Pb exposure levels that result in blood Pb levels >10 µg/dL. Evidence in animal studies also suggests the potential for increased BP following short-term (4 weeks) Pb treatment that included injected bolus doses that may have uncertain relevance to human routes of Pb exposure (Fiorim et al., 2011; Simões et al., 2011; Sharifi et al., 2004). These effects may be partially reversible; a study demonstrated partial reversibility (not to levels in controls) of Pb-induced elevations in BP following Pb exposure cessation or chelation (Chang et al., 2005).

Epidemiologic studies continued to investigate the relationship between bone Pb and BP. A recently published meta-analysis (Figure 4-22) (Navas-Acien et al., 2008) included several studies (three prospective, five cross-sectional) that individually showed that bone Pb level was associated with systolic BP but not diastolic BP. In the cross-sectional studies, a pooled estimate indicated an increase in systolic BP of 0.26 mmHg (95% CI: 0.02, 0.50) per 10 µg/g tibia Pb. In the longitudinal studies, a 0.33 mmHg (95% CI: -0.44, 1.11) increase was estimated per 10 µg/g bone Pb. Most studies also reported associations of bone Pb with hypertension. Pooled odds ratios for hypertension of 1.04 (95% CI: 1.01, 1.07) per 10 µg/g increase in tibia Pb and 1.04 (95% CI: 0.96, 1.12) per 10 µg/g increase in patella Pb were reported.



In the Normative Aging Study, Hu et al. (1996a) reported the cross-sectional association between bone Pb levels and the prevalence of hypertension and Cheng et al. (2001) reported the cross-sectional association between bone Pb levels and systolic BP in study participants free of hypertension at baseline.

Note: The studies are ordered by increasing mean bone Pb levels. The area of each square is proportional to the inverse of the variance of the estimated change or log relative risk. Horizontal lines represent 95% confidence intervals. Diamonds represent summary estimates from inverse-variance weighted random effects models. Because of the small number of studies, summary estimates are presented primarily for descriptive purposes. RR indicates risk ratio.

Source: Reprinted with permission of Lippincott Williams & Wilkins, Inc., Navas-Acien et al. (2008)

Figure 4-22 Meta-analysis of an increase in systolic BP (SBP) and diastolic BP (DBP) and relative risk of hypertension per 10 µg/g increase in bone Pb levels.

A few recent epidemiologic studies also emphasized the potential interaction between measures of long-term Pb exposure, i.e., bone Pb levels, and factors such as chronic stress and HFE genetic variants to moderate or modify the relationship of BP and hypertension with Pb. For example, among NAS men, tibia Pb level was associated with a larger risk of developing hypertension in an originally nonhypertensive group among men with higher self-reported stress (Peters et al., 2007).

In addition to stress, recent epidemiologic studies investigated effect modification by race/ethnicity and genetic variants. In the NHANES 1988-1994 population of adults, the

association of concurrent blood Pb with systolic BP was higher among Mexican Americans. In the same NHANES population, the association between blood Pb level and hypertension was higher among non-Hispanic Blacks with the ALAD2 allele (see Figure 4-17 and Figure 4-18 for results) (Scinicariello et al., 2010). Additionally, the association between blood Pb and PP was larger among NAS men with the HFE H63D variant (Figure 4-17) (Zhang et al., 2010a). PP represents a good predictor of cardiovascular morbidity and mortality and an indicator of arterial stiffness. The aforementioned genes are related to Fe metabolism and have been linked with differences in Pb distribution in blood and bone. Park et al. (2009a) provided further evidence of variants in Fe metabolism genes impacting the association of bone Pb levels with QT interval changes (see Table 4-21 for results).

Animal toxicological evidence continued to build on the evidence characterizing the mechanisms leading to these Pb-induced cardiovascular alterations. Biological plausibility for the consistent associations observed between blood and bone Pb and cardiovascular effects is provided by enhanced understanding of Pb-induced oxidative stress including NO inactivation, endothelial dysfunction leading to altered vascular reactivity, activation of the RAAS, and vasomodulator imbalance.

4.4.3 Vascular Effects and Cardiotoxicity

Not only has Pb been shown to increase BP and alter vascular reactivity, but Pb can alter cardiac function, initiate atherosclerosis, and increase cardiovascular mortality. Past toxicological studies have reported that Pb can increase atheromatous plaque formation in pigeons, increase arterial pressure, decrease heart rate and blood flow, and alter cardiac energy metabolism and conduction (Prentice and Kopp, 1985; Revis et al., 1981). A limited number of available epidemiologic studies discussed in the 2006 Pb AQCD (U.S. EPA, 2006b) provided evidence of associations of blood Pb level with ischemic heart disease (IHD) and peripheral artery disease (PAD).

4.4.3.1 Effects on Vascular Cell Types

The endothelial layer is an important constituent of the blood vessel wall, which regulates macromolecular permeability, VSMC tone, tissue perfusion, and blood fluidity. Damage to the endothelium is an initiating step in development of atherosclerosis, thrombosis, and tissue injury. Given that epidemiologic and toxicological evidence suggests that long-term Pb exposure is associated with a number of these conditions, numerous toxicological studies have investigated and found an effect of Pb on endothelial

dysfunction. A recent occupational study found that endothelial function assessed by flow-mediated dilatation was impaired in highly Pb-exposed workers (mean blood Pb levels: 24.1 µg/dL in workers versus 7.8 µg/dL in unexposed controls) (Poręba et al., 2010).

The endothelial layer makes up only a small part of the vascular anatomy; the majority of the vessel wall is composed of VSMCs, which work in concert with the endothelial cells (EC) in contraction and relaxation of the vessel, local BP regulation, and atherosclerotic plaque development. Since Pb has been shown repeatedly to result in hypertension and vascular disease in experimental animals, studies continued to investigate and find an effect of Pb on VSMCs.

In in vitro assays, Pb (50 µM, 2 weeks) stimulated VSMC invasiveness in isolated human arteries leading to the invasion of medial VSMC into the vessel intima and development of intimal hyperplasia, a key step in atherosclerotic progression (Zeller et al., 2010). In addition, treatment with Pb (50 µM, 12 hours) promoted VSMC elastin expression and increased arterial extracellular matrix in isolated human arteries. VSMC invasiveness was also increased in culture by treatment with supernatant of Pb-treated human EC (50 µM), suggesting that Pb-exposed ECs secrete an activating compound. This compound was confirmed to be IL-8. Pb exposure (5-50 µM) was able to, in a concentration-dependent manner, increase IL-8 synthesis and secretion in human umbilical vein EC cultures through activation of the transcription factor Nrf2. Neutralization of IL-8 could block VSMC invasion and arterial intima thickening (Zeller et al., 2010). This study provides evidence that Pb exposure stimulates ECs to secrete IL-8 in an Nrf2-dependent manner which stimulates VSMC invasion from the vessel media to intima leading to a vascular thickening and possibly atherogenesis.

A number of CVDs, including atherosclerosis, are characterized by increased inflammatory processes. Numerous studies have shown that Pb exposure is associated with an inflammatory environment in vascular tissues of humans and animals as indicated by higher levels of inflammatory mediators like prostaglandin E_2 (PGE_2). Human aortic VSMCs treated with Pb (1 µM, 1-12 hours) exhibited increased secretion of PGE_2 time-dependently through enhanced gene transcription (Chang et al., 2011). This was preceded by a Pb-induced increase in the gene expression of cytosolic phospholipase A_2 ($cPLA_2$) and COX-2, two rate limiting enzymes in the regulation of prostaglandins. The induction of these enzymes was mediated by activation of ERK1/2, MEK1, and MEK2. Further investigation of the entrance of Pb into the cell revealed that inhibition of the store-operated calcium channels (SOC) could only partially suppress $cPLA_2$ and COX activation by Pb; however, inhibition of epidermal growth factor receptor (EGFR) attenuated Pb-induced PGE_2 secretion and activation of $cPLA_2$ and COX. A follow-up to

this study found that Pb treatment (1μM) of a human epithelial cell line increased COX-2 gene expression, promoter activity, and protein (Chou et al., 2011). Inhibition of NF-κB decreased the Pb-induced COX-2 activation; whereas EGFR inhibition blocked COX-2 upregulation and NF-κB nuclear translocation. Overall these results suggest that Pb can induce pro-inflammatory events in VSMC in the form of increased PGE_2 secretion and expression of $cPLA_2$ and COX-2 through activation of EGFR via ERK1/2 and NF-κB pathways.

Damage to the endothelium is a hallmark event in the development of atherosclerosis. Past studies have shown that Pb exposure results in de-endothelialization, impaired proliferation, and inhibition of endothelium repair processes after injury (Fujiwara et al., 1997; Ueda et al., 1997; Kaji et al., 1995; Kishimoto et al., 1995). However, Pb exposure was not found to lead to nonspecific cytotoxicity at low exposure levels (2-25 μM) as shown by the lack of release of lactate dehydrogenase (LDH) from Pb-treated bovine aortic EC (Shinkai et al., 2010). Instead, Pb induced specific apoptosis of EC (caspase3/7 activation) through endoplasmic reticulum (ER) stress that was protected against by the ER chaperones glucose-regulated protein 78 (GRP78) and glucose-regulated protein 94 (GRP94). GRP78 and GRP94 play key roles in the adaptive unfolded protein response that serves as a marker of and acts to alleviate ER stress. Exposure of ECs to Pb induced *GRP78* and *GRP94* gene (2-25 μM) and protein (GRP78 [5-25 μM] and GRP94 [10-25 μM]) expression through activation of the IRE1-JNK-AP-1 pathways (Shinkai et al., 2010). This finding suggests that the functional damage in ECs caused by Pb exposure may be partly attributed to induction of ER stress.

4.4.3.2 Cholesterol

As blood cholesterol rises so does the risk of coronary heart disease. Previous occupational studies (Ademuyiwa et al., 2005a; Bener et al., 2001b; Kristal-Boneh et al., 1999) examining higher than most current adult blood Pb levels (>40 μg/dL) reported higher total cholesterol levels related to Pb exposure, but mixed results for HDL, LDL, and triglycerides. More recently, Poreba et al. (2010), in an occupational study, reported no significant differences in parameters of lipid metabolism between Pb exposed workers (mean blood Pb level: 25 μg/dL) and unexposed individuals. Conversely, Kamal et al. (2011) reported that occupational Pb exposure (mean blood Pb level: >40 μg/dL) was associated with higher levels of triglycerides, total cholesterol, and LDL, and decreased HDL-C. Other Pb studies adjusted models for total cholesterol to control for this coronary heart disease risk factor. Higher mean total cholesterol with higher blood Pb levels has been reported in an NHANES study (Menke et al., 2006). In developing models to predict bone Pb levels, Park et al. (2009b) noted in an NAS study that total and HDL

cholesterol were selected as 2 of 18 predictors for the bone Pb level model. Their findings suggested that higher Pb exposure in nonoccupationally-exposed men may be associated with higher total and HDL cholesterols. In support of epidemiologic evidence, a recent toxicological study reported increased LDL and decreased HDL in rats treated with Pb (20 ppm, i.p., 3 days/week, 8 weeks) (Roshan et al., 2011). The major risk factor that lipids represent for heart disease make relating lipid levels to Pb exposures an interesting but challenging hypothesis to test.

4.4.3.3 Atherosclerosis

A small number of toxicological and cross-sectional epidemiologic studies provide evidence for increased atherosclerosis and intimal medial thickening (IMT) due to Pb exposure. The association of stroke subtypes and severity of cerebral atherosclerosis was examined in relation to a single concurrent blood Pb level and total 72-hour urinary Pb level (body Pb store-EDTA mobilization test) in a cross-sectional study of 153 patients (mean age 63.7 years) receiving digital subtraction angiography in Chang Gung Memorial Hospital in Taiwan from 2002 to 2005 (Lee et al., 2009). In an analysis adjusted for age, sex, hypertension, diabetes, triglyceride, uric acid, smoking, and alcohol consumption, a 1 µg increase in urine Pb was associated with ≥ 50% stenosis in the intracranial carotid system with an OR of 1.02 (95% CI: 1.00, 1.03). Urine Pb was not associated with greater stenosis in the extracranial or vertebrobasilar systems. Blood Pb level was not associated with greater stenosis in any region. As the development of atherosclerosis is a lifelong process, body Pb stores, analyzed by total 72-hour urine Pb amount, may more strongly be associated with atherosclerosis than are single blood Pb measurements.

A recent study correlated greater carotid artery IMT with higher concurrent serum Pb levels (mean [SD] 0.41 [0.38] µg/dL) in hemodialysis patients (Ari et al., 2011). A few available recent occupational studies also presented evidence for increased measures of atherosclerosis in highly Pb-exposed adult populations with mean blood Pb levels around 25 µg/dL. Poręba et al. (2011a) reported increased local arterial stiffness and more frequent left ventricular diastolic dysfunction in Pb-exposed workers with hypertension compared to non-exposed controls with hypertension. Occupational exposure to Pb (mean blood Pb levels: 24 µg/dL in workers, 8.3 µg/dL in non-exposed group) was also associated with greater IMT and atherosclerotic plaque presentation, analyzed by Doppler ultrasound (Poreba et al., 2011).

Zeller et al. (2010) examined human radial and internal mammary arteries exposed to Pb in culture and reported a concentration-dependent increase in arterial intimal thickness

(statistically nonsignificant at 5 µM Pb, significant at 50 µM Pb, 2 week treatment) and intimal extracellular matrix accumulation (50 µM). Also, Pb promoted EC proliferation (5 and 50 µM, 72 hours) and VSMC elastin expression (50 µM, 12 hours), as discussed above (Section 4.4.3.1) (Zeller et al., 2010). Another study showed that Pb exposure (100 ppm in drinking water for 10 months; mean blood Pb level 28.4 µg/dL) of rats also increased the aortic media thickness, media-lumen ratio, and medial collagen content (Zhang et al., 2009a). These morphological changes to the vessel due to Pb exposure indicate initiation of arteriosclerosis and could be the cause of decreased contractile response of the vessel due to altered visco-dynamic vessel properties. Alternatively, these vascular changes could be an effect of Pb-induced hypertension.

4.4.3.4 Heart Rate Variability

HRV and BP are regulated, in part, by the sympathetic and parasympathetic nervous systems. Changes in either may increase the risk of cardiovascular events. HRV is defined as the oscillation in the interval between consecutive heart beats and between consecutive instantaneous heart rates. Decreases in HRV have been associated with cardiovascular mortality/morbidity in older adults and those with significant heart disease (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). In addition, decreased HRV may precede some clinically important arrhythmias, such as atrial fibrillation, as well as sudden cardiac death, in high risk populations (Chen and Tan, 2007; Sandercock and Brodie, 2006).

Pb has been shown not only to affect vascular contractility in animals, but also is associated with cardiac contractility. The 2006 Pb AQCD (U.S. EPA, 2006b) described one study that investigated Pb-induced alterations in HRV (Cheng et al., 1998). Cheng et al. (1998) found increasing duration of corrected QT interval (QTc) with increasing bone Pb levels in men <65 years, but not in men ≥ 65 years. Eum et al. (2011) and Park et al. (2009a) followed up on this previous NAS cohort (Cheng et al., 1998) (details found in Table 4-21). Eum et al. (2011) prospectively examined the association between blood and bone Pb levels and the development of electrocardiographic (ECG) conduction abnormalities among 600 men who were free of ECG abnormalities at the baseline assessment. A second ECG was obtained for 496 men 8.1 (SD: 3.1) years later on average. Baseline Pb concentrations in blood (mean [SD]: 5.8 [3.6] µg/dL), patella bone (mean [SD]: 30.3 [17.7] µg/g), and tibia bone (mean [SD]: 21.6 [12.0] µg/g) were similar to those found in other samples from the general U.S. adult population and much lower than those reported in occupationally exposed groups. Higher tibia Pb was associated with increases in QTc interval and QRSc duration. Compared with those in the lowest tertile of baseline tibia Pb (<16 µg/g), participants in the highest tertile (>23 µg/g) had a

7.94 msec (95% CI: 1.42, 14.45) greater increase in QTc interval and a 5.94 msec (95% CI: 1.66, 10.22) greater increase in QRSc duration over 8 years after adjusting for covariates: age, education, smoking, BMI, albumin-adjusted serum calcium, and diabetes status at baseline, and years between ECG tests and QT-prolongation drugs at the time of ECG measurement. There were no statistically significant associations with patella or blood Pb levels. These associations with tibia bone Pb levels were observed in men with relatively low blood and bone Pb concentrations who were free of cardiac conduction abnormalities at baseline and were examined prospectively. Thus, they indicate that long-term cumulative Pb exposure may increase the risk of developing cardiac abnormalities. Uncertainty exists as to the specific Pb exposure level, timing, frequency, and duration contributing these associations observed for tibia Pb levels. A recent occupational study reported lower HRV and abnormal parameters of heart rate turbulence in Pb-exposed workers (mean blood Pb levels: ~25 µg/dL) compared to control subjects (Poręba et al., 2011b).

Park et al. (2009a) cross-sectionally examined whether polymorphisms in genes known to alter Fe metabolism (HFE, transferrin [TF] C2, heme oxygenase-1 [HMOX-1]) modify the association between Pb biomarker levels and the QT interval. Investigators examined associations in data stratified on polymorphisms in the three genes. They also analyzed interaction models with cross-product terms for genotype and the Pb biomarker. The distributions of all genotypes but the HFE variant, H63D, were in Hardy-Weinberg equilibrium. Subjects homozygous for the other HFE variant, C282Y, had higher bone Pb levels and those homozygous for H63D and heterozygous with both C282Y and H63D had lower bone Pb levels. The antioxidant HMOX-1 L variant (longer repeats of GT, associated with lower enzyme inducibility) alone, compared to the wild type, showed a statistically significant interaction with tibia Pb (11.35 msec longer QTc interval for each 13 µg/g increase in bone Pb in L-allele variants). No other gene variant alone showed different Pb-associated QTc intervals from those in wild types, either for tibia and patella Pb or for (linear) concurrent blood Pb. Lengthening of QTc with higher tibia and blood Pb was more pronounced with an increase in the total number of gene variants, driven by a joint effect between HFE variants and HMOX-1 L allele. There was a trend observed with blood and tibia Pb-associated QTc interval increasing with increasing number of gene variants from 0 to 3. This study provided further evidence of gene variants modifying associations of Pb biomarkers with cardiovascular effects.

The interaction of key markers of the metabolic syndrome with bone Pb levels in affecting HRV was cross-sectionally investigated in a group of 413 older adults with patella Pb measurements in the NAS (Park et al., 2006). Metabolic syndrome was defined to include three or more of the following: waist circumference >102 cm, hypertriglyceridemia (>150 mg/dL), low HDL cholesterol (<40 mg/dL in men), high BP

>130/85 mmHg, and high fasting glucose (>110 mg/dL). Men using antihypertensive medication or diabetes medications were counted as high BP or high fasting glucose, respectively. The strongest relationships between patella Pb levels and lower HRV were observed among those with three or more metabolic abnormalities. A trend was observed for larger patella Pb-associated decreases in HRV with increasing number of metabolic abnormalities. These results suggest multiplicative effects of cumulative Pb exposure and metabolic abnormalities on key predictors of CVD. Park et al. (2006) also reported the penalized spline fits to bone Pb in models assessing only main effects of bone Pb. The optimal degree of smoothing determined by the generalized cross-validation criterion for all HRV measures was 1, which indicated that the associations were nearly linear. The spline fits and associated statistics showed that the bone Pb main effects on HRV measures were linear. However, the relationship with the LF/HF HRV measure was linear with log(LF/HF).

Increased incidence of arrhythmia and atrioventricular conduction block was found in rats after 12 weeks of Pb exposure (100 ppm; mean blood Pb level 26.8 µg/dL) (Reza et al., 2008). Also, Pb exposure for 8 weeks increased heart rate and systolic BP. These increases corresponded with increased cardiac contractile force and prolonged ST interval, without alteration in QRS duration or coronary flow. In contrast, another study using rat right ventricular strips found that Pb (100 µM) exposure, in a concentration-dependent manner, reduced myocardial contraction by reducing sarcolemmal Ca^{2+} influx and myosin ATPase activity (Vassallo et al., 2008). This study also found that Pb exposure changed the response to inotropic agents and blunted the force produced during contraction. Conversely, past studies have found that Pb exposure increases intracellular Ca^{2+} content (Lal et al., 1991; Favalli et al., 1977; Piccinini et al., 1977), which could result in increased cardiac output and hypertension.

Table 4-21 Characteristics and quantitative data for associations of blood and bone Pb with other CVD measures, i.e., HRV, PAD, and IHD in recent epidemiologic studies.

Study (Ordered as they appear in the text)	Study Population/ Methodology	Parameter	Pb Data	Statistical Analysis	Effect Estimate (95% CI)[a]
Heart rate variability					
Eum et al. (2011)	Longitudinal 600 elderly men (mostly white, mean age 67 yr) free of electrographic abnormalities at the time of baseline ECG enrolled in NAS in Greater Boston, MA area (496 with follow-up ECG 8 years later)	ECG conduction[b] (QTc, QRSc, JTc, QT prolongation, JT prolongation, IVCD[c], AVCD, Arrhythmia)	Baseline Blood Pb: Mean (SD): 5.8 (3.6) µg/dL Baseline Patella Pb: Mean (SD): 30.3 (17.7) µg/g Baseline Tibia Pb: Mean (SD): 21.6 (12.0) µg/g Q1: <16 µg/g (n = 191) Q2: 16.0 - 23 µg/g (n = 208) Q3: >23 µg/g (n = 195)	Repeated measures linear regression adjusted for age, education, smoking, BMI, albumin-adjusted serum Ca^{2+}, and diabetes status at baseline, years between ECG tests, and QT-prolongation drugs at the time of ECG measurement.	Tibia Pb: Adjusted 8-year change: QTc: Q2 vs. Q1 (reference): 7.49 (1.22, 13.75) msec, Q3 vs. Q1: 7.94 (1.42, 14.45) msec p for trend = 0.03 QRSc: Q2 vs. Q1: 0.52 (-3.60, 4.65) msec Q3 vs. Q1: 5.94 (1.66, 10.22) msec p for trend = 0.005 No associations with patella or blood Pb
Park et al. (2009a)	Cross-sectional 613 elderly men (mostly white, mean age 67 yr) enrolled in NAS in Greater Boston, MA area (8/1991 - 12/1995)	QTc[b] interval	Blood Pb: Median (IQR): 5 (4-7) µg/dL Patella Pb: Median (IQR): 26 (18-37) µg/g Tibia Pb: Median (IQR): 19 (14-27) µg/g	Linear regression models adjusted for age, BMI, smoking status, serum Ca^{2+}, and diabetes. No SES indicator was considered.	Per IQR (3 µg/dL) increase in blood Pb: 1.3 (-0.76, 3.36) msec after 8-year follow up Per IQR (19 µg/g) increase in patella Pb: 2.64 (0.13, 5.15) msec Per IQR (13 µg/g) increase in tibia Pb: 2.85 (0.29, 5.40) msec

Table 4-21 (Continued): Characteristics and quantitative data for associations of blood and bone Pb with other CVD measures, i.e., HRV, PAD, and IHD in recent epidemiologic studies.

Study (Ordered as they appear in the text)	Study Population/ Methodology	Parameter	Pb Data	Statistical Analysis	Effect Estimate (95% CI)[a]
Park et al. (2006)	Cross-sectional 413 elderly men (mostly white, mean age 67 yr) enrolled in NAS in Greater Boston, MA area (11/14/2000 - 12/22/2004)	HRV (SDNN, HF, HF_{norm}, LF, LF_{norm}, LF/HF)	Tibia Pb: Median (IQR): 19.0 (11-28) µg/g Patella Pb (measured within 6 mo of HRV: Median (IQR): 23.0 (15-34) µg/g Estimated[a]: Median (IQR): 16.3 (10.4-25.8) µg/g	Log linear regression models adjusted for age, cigarette smoking, alcohol consumption, room temperature, season (model 2) BMI, fasting blood glucose, HDL cholesterol, triglyceride, use of β-blockers, Ca^{2+} channel blockers, and/or ACE inhibitors. No SES indicator was considered.	Per IQR (17 µg/g) tibia Pb, Model 2 HF: –0.9 (–3.8, 2.1) normalized units (nu) LF: 0.9 (–2.0, 3.9) nu Log LF/HF: 3.3 (–10.7, 19.5) (%) Per IQR (15.4) µg/g estimated patella Pb, Model 2 HF: –0.6 (–3.1, 1.9) nu LF: 0.6 (–1.9, 3.1) nu Log LF/HF: 3.0 (–8.7, 16.2) (%) Effect estimates were more pronounced among those with greater # metabolic abnormalities.
Peripheral artery disease					
Muntner et al. (2005)	Cross-sectional 9,961 NHANES (1999-2002) participants	PAD	Range Concurrent Blood Pb: Q1: <1.06 µg/dL, Q2: 1.06-1.63 µg/dL Q3: 1.63-2.47 µg/dL Q4: >2.47 µg/dL	Logistic regression models adjusted for age, race/ethnicity, sex, diabetes mellitus, BMI, cigarette smoking, alcohol consumption, high school education, health insurance status	OR Q1: 1.00 (Reference), Q2: 1.00 (0.45, 2.22), Q3: 1.21 (0.66, 2.23), Q4: 1.92 (1.02, 3.61)

Table 4-21 (Continued): Characteristics and quantitative data for associations of blood and bone Pb with other CVD measures, i.e., HRV, PAD, and IHD in recent epidemiologic studies.

Study (Ordered as they appear in the text)	Study Population/ Methodology	Parameter	Pb Data	Statistical Analysis	Effect Estimate (95% CI)[a]
Navas-Acien et al. (2005)	Cross-sectional 790 participants, age ≥ 40 yr, from NHANES (1999-2000)	PAD	Concurrent urinary Pb: Mean (10th-90th percentile): 0.79 (0.2-2.3) µg/L	Logistic regression adjusted for the following: Model 1: age, sex, race, and education Model 2: covariates above plus smoking status Model 3:covariates above plus urinary creatinine	Per IQR increase in urinary Pb Model 1: OR: 1.17 (0.81, 1.69) Model 2: OR: 1.17 (0.78, 1.76) Model 3: OR: 0.89 (0.45, 1.78) Array of metals in urine also evaluated.

Table 4-21 (Continued): Characteristics and quantitative data for associations of blood and bone Pb with other CVD measures, i.e., HRV, PAD, and IHD in recent epidemiologic studies.

Study (Ordered as they appear in the text)	Study Population/ Methodology	Parameter	Pb Data	Statistical Analysis	Effect Estimate (95% CI)[a]
Ischemic Heart Disease					
Jain et al. (2007)	Longitudinal 837 elderly men (mostly white, mean age 67 yr) enrolled in NAS in Greater Boston, MA area (1991-2001)	IHD (MI or angina pectoris)	Baseline Blood Pb Mean (SD): Non-cases 6.2 (4.3) µg/dL; Cases 7.0 (3.8) µg/dL Baseline Patella Pb Mean (SD): Non-cases 30.6 (19.7) µg/dL; Cases 36.8 (20.8) µg/dL Baseline Tibia Pb Mean (SD): Non-Cases 21.4 (13.6) µg/g; Cases 24.2 (15.9) µg/g Cases: Blood Pb range: 1.0 to 20.0 µg/dL Patella Pb range: 5.0 to 101 µg/g Tibia Pb range: -5 to 75 µg/g	Cox proportional hazards models adjusted for age, BMI, education, race, smoking status, pack-years smoked, alcohol intake, history of diabetes mellitus and hypertension, family history of hypertension, DBP, SBP, serum triglycerides, serum HDL, and total serum cholesterol	Blood Pb level ≥ 5 µg/dL Per 1 SD increase in Pb biomarker OR over 10-year follow-up: 1.73 (1.05, 2.87) Ln [blood Pb] OR: 1.45 (1.01, 2.06) Ln [patella Pb level] OR: 2.64 (1.09, 6.37) Ln [tibia Pb level] OR: 1.84 (0.57, 5.90)

[a]Estimated patella Pb accounts for declining trend in patella Pb levels between analysis of bone Pb and HRV.

[b]Heart-rate-corrected QT interval calculated by Bazett's formula

[c]IVCD, intraventricular conduction defect; AVCD, atrioventricular conduction defect

4.4.3.5 Peripheral Artery Disease

Peripheral artery disease (PAD) is an indicator of atherosclerosis and measured by the ankle brachial index, which is the ratio of BP between the posterior tibia artery and the brachial artery. PAD is typically defined as an ankle brachial index of less than 0.9. Muntner et al. (2005), whose results describing the association of blood Pb and hypertension in the NHANES 1999-2002 data set for adults were discussed previously, also examined the association of blood Pb with PAD (details found in Table 4-21). The authors observed an increasing trend in the odds of PAD with increasing concurrent blood Pb level. The OR for PAD comparing the fourth quartile of blood Pb (>2.47 µg/dL) to the first quartile of blood Pb (<1.06 µg/dL) was 1.92 (95% CI: 1.02, 3.61). Key potential confounding factors Table 4-21) were adjusted for in the analysis. These results are consistent with those from a previous NHANES analysis by Navas-Acien et al. (2004) reviewed in the 2006 Pb AQCD.

Navas-Acien et al. (2004) reported a trend of increasing OR for PAD with increasing quartile of concurrent blood Pb or Cd in adults who were 40 years of age in the 1999-2000 NHANES population. These authors tested both Pb and Cd in separate models, tested the metals simultaneously, and tested the interaction between the metals. The correlation coefficient between natural log Pb and natural log Cd was 0.32 ($p < 0.001$). Although the interaction was not statistically significant, when blood Pb and blood Cd were in the same model, the ORs were diminished slightly. Both showed statistically significant trends of increasing OR with increasing quartile of the metal. These results indicate that blood Cd levels did not confound the association between blood Pb level and PAD. In a subsequent analysis, Navas-Acien et al. (2005) used the same 1999-2000 NHANES dataset, but constructed PAD models using a suite of urine metal concentrations. Power was reduced in this study because only 659-736 subjects (compared to 2,125) had spot urine metal tests in the data set. Urinary Cd, but not urinary Pb, was consistently associated with PAD in all models. Associations also were observed with urinary antimony and tungsten. Spot urine Pb measurements are less reliable compared to blood Pb measurements (Section 3.3.3). In Navas-Acien et al. (2005), the urinary Pb level association with PAD was sensitive to adjustment for urinary creatinine, indicating that spot urine Pb measurements are affected by differences in urine dilution. This finding illustrates the limited reliability of spot urine Pb measurements compared to blood Pb measurements.

4.4.3.6 Ischemic Heart Disease

A few cross-sectional studies discussed in the 2006 Pb AQCD (U.S. EPA, 2006b) indicated associations between Pb biomarker levels and increased risk of cardiovascular outcomes associated with IHD, including left ventricular hypertrophy (Schwartz, 1991) and myocardial infarction (Gustavsson et al., 2001). Recently, Jain et al. (2007) provided prospective evidence for the incidence of IHD (physician confirmed MI, angina pectoris) among older adult males enrolled in the NAS who were followed during the period of September, 1991 to December, 2001 (details found in Table 4-21). All subjects had blood Pb and bone Pb measurements with no IHD at enrollment. Fatal and nonfatal cases were combined for analysis. Baseline blood, tibia, and patella Pb levels were log-transformed. Blood Pb level and patella Pb level were associated with increased risk of IHD over the 10-year follow-up period. When blood Pb and patella Pb were included simultaneously in the model, each of their hazard ratios (HRs) was only moderately attenuated (HR: 1.24 [95% CI: 0.80, 1.93] per SD increase in blood Pb and HR: 2.62 [95% CI: 0.99, 6.93] per SD increase in patella Pb). When blood Pb and tibia Pb were included simultaneously in the model, their risk estimates were only moderately attenuated (HR: 1.38 [95% CI: 0.89, 2.13] per SD increase in blood Pb and HR: 1.55 [95% CI: 0.44, 5.53] per SD increase in tibia Pb). These findings indicate that both blood and bone Pb levels are independently associated with IHD incidence.

IHD, characterized by reduced blood supply to the heart, may result from increased thrombosis. In support of the epidemiologic evidence, a recent animal study suggested that Pb exposure promotes a procoagulant state that could contribute to thrombus formation (Shin et al., 2007). In a rat model of venous thrombosis, Pb treatment (i.v. 25 mg/kg) resulted in increased thrombus formation, although i.v. Pb treatment may have uncertain relevance to human routes of Pb exposure. Additionally, Pb treatment to human erythrocytes (red blood cells, RBCs) increased coagulation at a dose of 5 µM and thrombin generation in a concentration-dependent manner at doses from 2-5µM. This enhanced procoagulant activity in Pb-treated RBCs was the result of increased outer cell membrane phosphatidylserine (PS) surfacing (human RBCs: 2-5 µM Pb; rat RBCs: 5 µM Pb). Similar to these in vitro results, PS externalization on erythrocytes was increased in Pb-treated rats (i.v. 50-100 mg/kg, not 25 mg/kg). Increased PS externalization was likely the result of increased intracellular calcium (5 µM Pb), enhanced scramblase activity (5-10 µM Pb), inhibited flippase activity (5-10 µM Pb), and ATP depletion (1-5 µM Pb) after Pb exposure (Shin et al., 2007).

4.4.3.7 Summary of Vascular Effects and Cardiotoxicity

There are a limited number of studies in a limited number of populations that investigate the associations between Pb biomarkers and cardiotoxicity and cardiovascular effects other than BP or hypertension (Table 4-21). As presented in Table 4-21, these studies demonstrated associations between various biomarkers of Pb exposure and clinical cardiovascular outcomes such as atherosclerosis, IHD, PAD, and HRV occurrence in adult populations after adjusting for potential confounding by variables such as age, sex, education, BMI, smoking, alcohol consumption, and diabetes. In a limited body of studies, mixed evidence of association between occupational exposure to Pb and altered cholesterol was reported.

A limited number of studies have evaluated markers of subclinical atherosclerosis such as PAD (i.e., ankle-brachial index) and generalized atherosclerosis (i.e., IMT) following Pb exposure in humans or animals. Concurrent blood Pb levels (population means >2.5 µg/dL) were associated with greater odds of PAD in adults in NHANES analyses (Muntner et al., 2005; Navas-Acien et al., 2004). Since these effects were observed in adults that may have had higher past exposure to Pb, there is uncertainty as to the specific Pb exposure level, timing, frequency, and duration that contributed to the observed associations. A recent study involving both human and toxicological studies observed Pb-mediated arterial IMT, an early event in Pb-induced atherogenesis (Zeller et al., 2010). A second study in rats reports increased aortic media thickness following Pb exposure (Zhang et al., 2009a). Toxicological studies of Pb-induced endothelial dysfunction, VMSC invasiveness, and inflammation in isolated vascular tissues and cells provide mechanistic evidence to support the biological plausibility of these vascular effects and cardiotoxicity. Studies in isolated tissues and cells found that Pb stimulated the synthesis and secretion of IL-8 in ECs, which was responsible for stimulating VSMC invasion into the vessel intimal layer. Pb treatment also increased extracellular matrix and elastin, primary sites for lipid deposition in the vessel wall.

Several studies of the NAS cohort report associations between biomarkers of Pb exposure and diseases associated with coronary heart disease (CHD), such as HRV, IHD, and MI. A prospective NAS study reported that higher baseline tibia Pb was associated with increases in QTc interval and QRSc duration over an 8-year follow-up period (Eum et al., 2011). In addition, in the NAS cohort of older adult men, blood Pb (≥ 5 µg/dL) and patella Pb levels were associated with increased incidence of IHD (Jain et al., 2007). A recent study provided evidence for the interaction between biomarkers of Pb exposure and the HFE C282Y and HMOX-1 L variants on the prolonged QT interval in nonoccupationally-exposed older men (Park et al., 2009a). Also, in the NAS population, bone Pb levels were associated with larger decreases in HRV parameters among subjects

identified as having metabolic abnormalities (Park et al., 2006). These metabolic abnormalities, abdominal obesity, hypertriglyceridemia, low HDL cholesterol, high BP/medication use, or high fasting glucose, have been shown to be associated with increased risk of cardiovascular events.

Overall, the relatively few available studies provide support for associations between Pb biomarkers and other cardiovascular conditions including subclinical atherosclerosis and CHD. A number of these are quality studies from two cohorts, NAS and NHANES with adequate sample size that account for potential confounding, with some being conducted prospectively.

4.4.4 Cardiovascular Function and Blood Pressure in Children

4.4.4.1 Introduction

The study of cardiovascular function effects in relation to blood Pb levels in children potentially offers unique information on several topics. First, this research examines endpoints that may be predictive of future cardiovascular pathology, and consequently may offer information on the potential cardiovascular effects of Pb exposure in an understudied population, children, in whom cardiovascular events are not likely. Second, examination of cardiovascular changes that may be antecedent to later-in-life effects, such as increased BP and changes in other CVD-related endpoints, may inform the understanding of the time course of cardiovascular changes associated with Pb exposure. Finally, these studies address gaps in knowledge regarding Pb exposure effects in populations of children (ages 5-15 years and 19-29 years) with mostly concurrent mean blood Pb levels in the range of 1-5 µg/dL.

An important aspect to the literature about the association between cardiovascular effects and blood Pb levels in children is that the blood Pb levels of children may better reflect relatively recent Pb exposure and its effect on CVD than blood Pb levels do in adults because of the much longer exposure history of adults during which Pb exposures were commonly much higher than they are today. However, in older children there is still uncertainty regarding the frequency, duration, timing, and magnitude of exposure contributing to the blood Pb levels measured. The much lower prevalence of cardiovascular effects in children, however, poses a challenge to investigations of potential relationships with Pb exposures. For example, the prevalence of hypertension in children (9 to 10 years old) ranges from to 2 to 5 percent (Daniels, 2011; Steinthorsdottir et al., 2011), while more than half of people aged 60 to 69 years have hypertension (Chobanian et al., 2003). Accordingly, much larger study populations are required to

provide similar statistical power for such studies in children as compared to adult studies. Further, in drawing interpretations from such studies with regard to potential effects of Pb exposures at later ages, it is additionally important to recognize that compensatory mechanisms in children may be more active than in adults, and the cardiovascular tissue of the young may be less susceptible to damage than that of adults.

The limited numbers of cardiovascular studies published on children have examined endpoints such as total peripheral resistance (TPR), BP, and autonomic nervous system activation. These recent and earlier studies are presented in Table 4-22. Multiple single pollutant studies in New York State evaluated two child cohorts born in the 1990s after Pb was removed from gasoline in the U.S. with mean blood Pb levels of 4.62 and 1.01 µg/dL (Gump et al., 2011; Gump et al., 2009; Gump et al., 2007; Gump et al., 2005). The cohort examined in Gump et al. (2009; 2007; 2005) was a subset of the Oswego Children's Study designed as a prospective, prenatal polychlorinated biphenyls study. Gump et al. (2005) reported cardiovascular outcomes associated ($p<0.2$) with other prenatal toxicants (i.e., mercury, dichlorodiphenyldichlorethylene, hexachlorobenzene); however, the effects of postnatal and prenatal Pb were found even after covariate control for all non-Pb toxicants that were related to the outcome. Zhang et al. (2011a) examined children in Mexico City born from 1994 to 2003, when Pb was being taken out of gasoline in Mexico as indicated by Martinez et al. (2007). The geometric means for cord and concurrent blood Pb levels of the children (ages 7-15 years) in the Mexico City cohort were 4.67 and 2.56 µg/dL, respectively.

Table 4-22 Studies of child cardiovascular endpoints and Pb biomarkers.

Study (Ordered as they appear in the text)	Study Population/ Methodology	Parameters	Blood Pb Data[a]	Statistical Analysis	Effect Estimates/Results
Gump et al. (2005)	Prospective 122 children age 9.5 yr in Oswego, NY (born at a single hospital in New York from 1991-94)	SBP, DBP, SV (stroke volume), CO (cardiac output), TPR (total peripheral vascular resistance)	Cord blood Pb: GM (GSD): 2.56 µg/dL (1.16) Childhood (mean age of measurement: 2.6 yr) blood Pb: GM (GSD): 4.06 µg/dL (1.14) MDL: 1 µg/dL	Multivariate linear regression models examined the relationship of blood Pb with change in z-score for outcome (post- and pre-stress). Potential confounders considered: HOME score, SES, birth weight, child BMI, child sex.	Per 1 µg/dL increase in childhood blood Pb level: TPR: 0.088 (95% CI: 0.023, 0.153) dyne-s/cm^5 SV:-0.069 (95% CI: -0.124, -0.015) mL CO: -0.056 (95% CI: -0.113, 0.001) L/min DBP: 0.069 (95% CI: -0.001, 0.138) mmHg Per 1 µg/dL increase in cord blood Pb level: SBP: 12.16 (95% CI: 2.44, 21.88) mmHg Blood Pb levels were not associated with baseline cardiovascular levels.

Table 4-22 (Continued): Studies of child cardiovascular endpoints and Pb biomarkers.

Study (Ordered as they appear in the text)	Study Population/ Methodology	Parameters	Blood Pb Data[a]	Statistical Analysis	Effect Estimates/Results
Gump et al. (2007)	Prospective 122 children age 9.5 yr in Oswego, NY	SBP, TPR	Childhood (mean age of measurement: 2.6 yr) blood Pb: GM (GSD): 4.06 µg/dL (1.14)	Linear regression models adjusting for the same covariates as in Gump et al. (2005). Separate models testing whether Pb is a mediator of SES associations (Sobel test) and whether Pb moderates SES associations (Pb-SES interaction).	Blood Pb was a mediator of the SES-TPR relationship SES alone: -0.62 dyne-s/cm^5 ($p < 0.05$) SES with Blood Pb: -0.40 dyne-s/cm^5 ($p > 0.10$), change in R^2 attributable to SES: -55.3% Blood Pb was a potential moderator of the SES-TPR relationship. Blood Pb × SES interaction: p = 0.07 Blood Pb was a moderator of SES-SBP relationship Pb × SES interaction: p = 0.007 At blood Pb levels >4 µg/dL, SES not significantly associated with SBP
Gump et al. (2009)	Prospective 122 children age 9.5 yr in Oswego, NY	Salivary cortisol	Cord blood Pb: GM (GSD): 2.56 µg/dL (1.16) Childhood (mean age of measurement: 2.6 yr) blood Pb: GM (GSD): 4.06 µg/dL (1.14)	Linear regression to examine whether blood Pb level mediates or moderates the relationship between SES and salivary cortisol as in Gump et al. (2007)	Blood Pb was a mediator of the SES-cortisol association. SES was no longer significantly associated with cortisol after adjusting for blood Pb level. R^2 for SES decreased by 40, 33, and 50% for cortisol measured at 21, 40, and 60 min. Blood Pb was not a significant moderator of SES-cortisol association. Blood Pb × SES interaction term was not statistically significant

Table 4-22 (Continued): Studies of child cardiovascular endpoints and Pb biomarkers.

Study (Ordered as they appear in the text)	Study Population/ Methodology	Parameters	Blood Pb Data[a]	Statistical Analysis	Effect Estimates/Results
Gump et al. (2011)	Cross-sectional 140 children ages 9-11 yr Oswego, NY	SBP, TPR, HRV (heart rate variability) in response to acute stress (mirror tracing task)	Concurrent blood Pb: GM: 1.01 µg/dL Quartiles: Q1: 0.14-0.68 µg/dL Q2: 0.69-0.93 µg/dL Q3: 0.94-1.20 µg/dL Q4: 1.21-3.76 µg/dL MDL: 0.28-0.34 µg/dL	Outcomes were analyzed as continuous variables for the pre-stress values or the change post- and pre-stress. Regression models were adjusted for sex, SES, BMI, and age.	Blood Pb levels associated with autonomic and cardiovascular dysregulation in response to stress – greater vascular resistance, reduced stroke volume, and cardiac output Change in SBP (mmHg) across quartiles: Q1: 5.30, Q2: 7.33, Q3: 7.07, Q4: 7.23, p for trend = 0.31 Change in TPR (%) across quartiles: Q1: 2.91, Q2: 8.18, Q3: 9.55, Q4: 9.51, p for trend = 0.03 Change in Stroke Volume (%) across quartiles: Q1: 2.23, Q2: 0.91, Q3: -3.47, Q4: -0.89, p for trend = 0.04 Blood Pb levels were not associated with baseline cardiovascular levels.
Zhang et al. (2011a)	Prospective 457 mother child pairs in a birth cohort, born 1994 to 2003 in Mexico City. Children were evaluated 2008-2010 at ages 7-15 yr	SBP	Cord blood Pb: GM (GSD): 4.67 µg/dL (1.18) (N=323) Concurrent blood Pb: GM (GSD): 2.56 µg/dL (1.16) (N=367) Maternal post-partum bone Pb: Median (IQR): Tibia Pb: 9.3 (3.3, 16.1) µg/g Patella Pb: 11.6 (4.5, 19.9) µg/g	Multiple regression models and generalized estimating equations (log linear for cord blood, linear for concurrent blood and maternal bone). The base model considered maternal education, birth weight, BMI, sex, and child concurrent age as covariates.	Prenatal Pb exposure may be associated with higher BP in female offspring. Among girls, an IQR (13 µg/g) increase in maternal tibia Pb was associated with a 2.11 (95% CI: 0.69, 3.52) mmHg increase in SBP IQR (16 µg/g) increase in maternal patella Pb was associated with a 0.87 (95% CI: -0.75, 2.49) mmHg increase in SBP IQR (4 µg/dL) increase in cord blood Pb was associated with a 0.75 (95% CI: -1.13, 2.63) mmHg increase in SBP

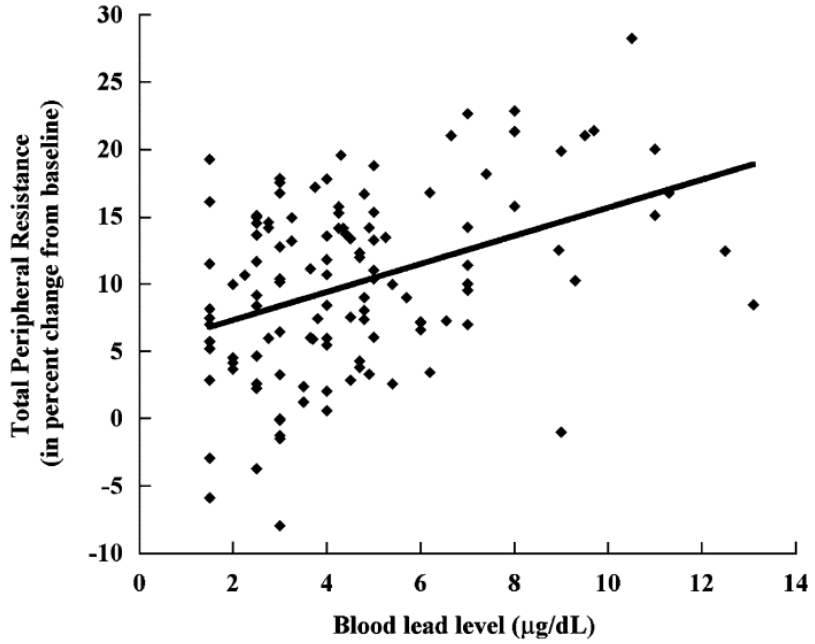
Table 4-22 (Continued): Studies of child cardiovascular endpoints and Pb biomarkers.

Study (Ordered as they appear in the text)	Study Population/ Methodology	Parameters	Blood Pb Data[a]	Statistical Analysis	Effect Estimates/Results
Factor-Litvak et al. (1999; 1996)	Cross-sectional 260 children ages 5.5 yr in K. Mitrovica and Pristina, Yugoslavia	SBP	Concurrent blood Pb range: 4.1 to 76.4 µg/dL	Linear regression analysis. Potential confounders considered: sex, maternal education, birth weight, HOME score, and BMI.	Per 1 µg/dL increase in concurrent blood Pb level: 0.05 (95% CI: -0.02, 0.13) mmHg higher SBP Blood Pb level at birth and cumulative blood Pb level were not as strongly associated with SBP at age 5.5 yr.
Gerr et al. (2002)	Cross-sectional 508 young adults age 19-29 yr, born 1965-1975, male and female; half of the subjects had grown up around an active Pb smelter in Silver Valley, Idaho	BP	While the concurrent mean blood Pb level was 3.15 µg/dL for the highest bone Pb category (>10 µg/g), early childhood mean blood Pb levels in this group were substantially elevated for all bone Pb level categories and were highest among participants in the highest bone Pb level category. The mean blood Pb level was 65 µg/dL among participants with bone Pb level >10 µg/g. Bone Pb was measured at the time of entry into this study.	Multiple linear regression models always included age, sex, height, BMI, current smoking status, frequency of alcohol consumption, current use of birth-control medication, hemoglobin level, serum albumin, and income, regardless of significance levels. Both blood Pb (as a linear term) and bone Pb (a four category ordinal variable from <1 µg/g to >10 µg/g) were tested together.	Group in highest quartile of tibia Pb level (>10 µg/g) had 4.26 (95% CI: 1.36, 7.16) mmHg higher SBP and 2.80 (95% CI: 0.35, 5.25) mmHg higher DBP compared to the lowest tibia Pb group (<1 µg/g).

[a]Blood Pb data are estimates of geometric mean (GM) and geometric standard deviation (GSD) using the arithmetic mean and SD.

4.4.4.2 Cardiovascular Functioning in Children

The relationship between cardiovascular functioning (TPR, BP, stroke volume, and cardiac output,) and blood Pb levels was examined prospectively by Gump et al. (2007; 2005) in a cohort derived from the Oswego Children's Study discussed in Section 4.4.4.1 and born at a single New York hospital. Higher early childhood Pb levels (average age 2.6 years) were associated with greater TPR response to acute stress induced by mirror tracing on a computer at age 9.5 years as shown in Figure 4-23. Testing blood Pb with linear, quadratic, and cubic terms did not produce significantly different Pb-TPR associations, and the authors suggested that these effects were concentration-dependent and notably, were not emergent at a specific exposure threshold. TPR increased with increasing quartile of blood Pb level. A mediational analysis indicated that Pb was a significant mediator of the SES-TPR reactivity association; some evidence also suggested moderation, whereby the inclusion of blood Pb into the model reduced the effect estimate for SES. Observations that Pb exposure increases TPR in toxicological studies and mechanistic evidence indicating that Pb-induced changes in SNS activity may mediate such effects (Section 4.4.2.3) provide some biological plausibility for a role of Pb in affecting the TPR response to acute stress in this child population. Additionally, higher early childhood blood Pb level (average age 2.6 years) was associated with a smaller stroke volume and cardiac output responses to acute stress at age 9.5 years (Gump et al., 2007). In a further analysis in this cohort, Gump et al. (2009) examined the possibility that Pb may mediate an association between SES and cortical responses to acute stress. Elevated cortisol has been associated with hypertension (Whitworth et al., 2000). Gump et al. (2009) found that lower family income was associated with greater cortisol levels following an acute stress task and that blood Pb was a mediator of this association, such that after controlling for early childhood blood Pb levels, family income was no longer predictive of cortisol levels.



Source: Reprinted with permission of Elsevier (Gump et al., 2005)

Figure 4-23 Children's adjusted total peripheral resistance (dyn-s/cm^5) responses to acute stress tasks, as a function of early childhood Pb levels.

In a different cohort of 140 children 9 to 11 years of age recruited from local pediatrician offices and from mailings to homes with children in this age group, Gump et al. (2011) used a similar acute stress-producing paradigm as in previous studies to examine the cross-sectional associations of concurrent blood Pb with cardiovascular responses. TPR significantly increased in a concentration-dependent relationship with blood Pb, with most of the increase occurring between the first quartile blood Pb (0.14-0.68 µg/dL) and the second quartile blood Pb (0.69-0.93 µg/dL). The first quartile contained samples from children with blood Pb levels below the method detection limit (MDL) (0.28 - 0.34 µg/dL). These values were used in the analyses and may be subject to greater measurement error; however, the error is likely nondifferential. This result is consistent with those of Gump et al. (2005). Also, these newer findings provided evidence of associations with concurrent blood Pb levels and with lower blood Pb levels (Gump et al., 2011) than were previously examined by Gump et al. (2005) and in a large group of children without higher Pb exposures earlier in childhood.

Studies in adults and animals indicate Pb-associated decreases in HRV (Section 4.4.3.4). In Gump et al. (2011), cardiac autonomic regulation decreased in a

concentration-dependent manner with increasing concurrent blood Pb quartile, with the largest change relative to the first quartile (0.14-0.68 µg/dL) measured in the highest blood Pb quartile (1.21-3.76 µg/dL). Also, high frequency HRV decreased more with acute stress in the highest Pb quartile group (1.21-3.76 µg/dL). In the earlier cohort, early childhood (mean age at collection: 2.6 years) blood Pb level was associated with reduced stroke volume and cardiac output measured at the age of 9.5 years (Gump et al., 2005). Blood Pb levels in Gump (2005) were determined in either venous (80%) or capillary (20%) blood specimens. Capillary blood Pb determinations have greater potential for contamination during collection resulting in greater measurement error, particularly at concentrations approaching the MDL (1 µg/dL). In a recent study, Gump et al. (2011) also found reduced stroke volume and cardiac output but for concurrent (mean age: 10.2 years) blood Pb level and at lower blood Pb levels.

4.4.4.3 Blood Pressure in Children

Zhang et al. (2011a) conducted a longitudinal study that examined changes in BP in 323 girls and boys aged 7 to 15 years in a Mexico City cohort and associations with maternal bone Pb measured one month post-partum (a measure of cumulative exposure that could expose fetuses to Pb through accelerated mobilization of bone Pb during pregnancy) and with cord blood Pb at delivery. This was the first study to examine the association of maternal bone Pb, as a marker of prenatal exposure, with offspring BP. The model including both girls and boys (without adjustment for concurrent blood Pb) showed no statistically significant association overall for any Pb biomarker with child BP. A significant interaction was found between maternal tibia Pb and sex, and in models stratified by sex, maternal tibia Pb was associated with adjusted systolic and diastolic BP in females, but not males. Maternal post-partum median tibia Pb was 9.3 µg/g (IQR: 3.3, 16.1 µg/g) with no significant differences between mothers of male and female offspring. Suboptimal growth in utero is associated with accelerated weight gain in offspring during childhood and greater risk of later hypertension (Barker and Bagby, 2005; te Velde et al., 2004; Barker et al., 1989). The relationship between birth weight and Pb biomarkers is discussed in Section 4.8.2. These may represent biologically plausible mechanisms by which prenatal Pb exposure may result in increased BP later in childhood as was demonstrated in female offspring.

Gump et al. (2011; 2005) examined the relationship of blood Pb level with BP in their two cohorts of contemporary children around age 10 years in New York State. Gump et al. (2005) reported an association of cord blood levels with systolic BP (12.16 mmHg [95% CI: 2.44, 21.88] increase per 1 µg/dL increase in cord blood Pb level). Gump et al. (2005) also reported cardiovascular outcomes associated ($p<0.2$) with other prenatal

toxicants (i.e., mercury, DDE, HCB); however, the effects of postnatal and prenatal Pb were found even after covariate control for all non-Pb toxicants that were related to the outcome. Gump et al. (2011) found that with acute stress, children in higher quartiles of concurrent blood Pb level (>0.69 µg/dL) had larger increases in systolic BP. For example, children with concurrent blood Pb levels between 1.21 and 3.76 µg/dL had a 7.23 mmHg change, and children with blood Pb levels between 0.14 and 0.68 µg/dL had a 5.30 mmHg change. A linear trend was not observed across quartiles. Blood Pb levels were not significantly associated with baseline cardiovascular levels. An interaction between long-term perceived stress and bone Pb levels in association with BP and hypertension also was reported in a study of adults (Peters et al., 2007) (described in Section 4.4.2.1). An earlier study (Factor-Litvak et al., 1999; Factor-Litvak et al., 1996) of children with higher blood Pb levels ranging from 4.1 to 76.4 µg/dL found that a 1 µg/dL increase in concurrent blood Pb was associated with a 0.05 (95% CI: -0.02, 0.13) mmHg increase in systolic BP. An additional study (Gerr et al., 2002) reported that systolic BP for young adults (ages 19-29 years) with bone Pb levels greater than 10 µg/g (mean concurrent blood Pb = 65 µg/dL) was 4.26 mmHg higher (diastolic BP was 2.8 mmHg greater) compared with young adults with bone Pb levels < 1 µg/g.

The pathogenesis of CVD has been hypothesized to begin in childhood (Kapuku et al., 2006). Early markers observable in youth in association with Pb biomarkers include increased BP during stress, reduced HRV, increased IMT, and vascular endothelium dysfunction. Kapuku et al. (2006) state that endothelial dysfunction is the center of the CVD paradigm. The factors measured in childhood or as a cumulative burden since childhood are predictors of outcomes in young adults who are still too young to experience coronary events (Li et al., 2003), and early-life exposures may induce changes in arteries that contribute to the development of atherosclerosis (Raitakari et al., 2003). Berenson (2002) observed that the effects of multiple risk factors on coronary atherosclerosis support evaluation of cardiovascular risk in young people. Thus, evidence relating levels of biomarkers of Pb exposure in children to cardiovascular function in the groups of studies presented in the preceding text when combined with the evidence for the potential pathogenesis of CVD starting in childhood that yield effects in adulthood, provides coherence with evidence in adults supporting the effects of long-term, cumulative Pb exposures in the development of cardiovascular effects.

Few animal studies have examined the effect of Pb exposure during pregnancy and lactation on BP in offspring as adults and those that have used high levels of exposure. Recently, pups of Pb-exposed dams (1,000 ppm through pregnancy and lactation) exhibited increased blood Pb level (mean blood Pb level: 58.7 µg/dL) and increased arterial systolic BP after weaning (Grizzo and Cordellini, 2008) suggesting a role for childhood-Pb exposure leading to adult disease.

4.4.4.4 Summary of Child Cardiovascular Studies

The 2006 Pb AQCD (U.S. EPA, 2006b) described three studies on the effects of Pb on cardiovascular function in children; however, no conclusions were made as to the strength of the evidence. Studies have reported cardiovascular changes antecedent to CVD such as TPR responses to acute stress tasks as a function of childhood blood Pb levels. Also, a study reported associations with acute stress-induced autonomic and cardiovascular dysregulation responses. Biomarkers of prenatal Pb exposure (maternal postpartum patella and tibia Pb levels) were related to later higher BP. Other lines of evidence have linked increased intrauterine growth restriction to later accelerated weight gain in childhood, and this may indicate greater risk of hypertension later in life. The results are not uniform with respect to the important lifestages of Pb exposure and can differ by sex and other factors. Uncertainties in these studies may be related to sample size, single measures of BP, variation in the age of onset of puberty, and cross-sectional design. However, some of these uncertainties may result in the attenuation of observed associations rather than the generation of spurious associations. Overall, recent study findings indicate that in children (ages 5-15 years) with mostly concurrent mean blood Pb levels of 1-5 µg/dL, increasing blood Pb level may be associated with small increases in BP and changes in the cardiovascular system. It is uncertain to what extent these associations relate to later-in-life effects, such as increased BP and the development of CVD.

Factors may limit the ability of studies to detect statistically significant Pb-associated changes with BP. The relatively young age of the subjects may have limited the ability of these studies to detect significant BP effects (as opposed to early function effects) if longer duration Pb exposure is necessary to produce the cardiovascular changes and considering the lower prevalence and strength of compensatory mechanisms in children. There is uncertainty in the shape of the concentration-response relationship for cardiovascular endpoints at lower blood Pb levels since most studies modeled a linear relationship. A nonlinear concentration-response relationship has been found for Pb with other outcomes in children, most notably, decrements in cognitive function (see Section 4.3.2).

Cardiovascular endpoints other than baseline BP may be more sensitive outcomes for measuring Pb-associated cardiovascular effects in young children. The series of studies by Gump et al. (2011; 2009; 2007; 2005) evaluating much smaller samples than did the adult studies, was able to demonstrate statistically significant relationships of blood Pb levels with cardiovascular responses such as TPR, related to acute stress. These results suggest that the stress paradigm may be useful to detect associations of blood Pb levels with effects on the cardiovascular system of children. Selection of the appropriate

cardiovascular outcome in children is an important factor to consider. Rather than using indicators of cardiovascular effects, such as BP, evaluation of cardiovascular changes that are antecedent to increased BP and changes in other CVD-related endpoints that present at later lifestages may be informative to understanding the time course of cardiovascular changes potentially associated with early Pb exposure.

Overall this small body of evidence, based on different cohorts, locations, and study designs, is suggestive of a relationship between biomarkers of Pb exposure and cardiovascular effects in children. One longitudinal study ties in maternal bone Pb level, and cord and concurrent blood Pb level for the children. Limitations exist in the studies. While BP increases are more prevalent in older adults than in children, BP increases have been related to higher blood Pb level in earlier studies of children and young adults (Gerr et al., 2002; Factor-Litvak et al., 1999; Factor-Litvak et al., 1996). The recent Gump studies provide information in populations of children (ages 9-11) with mean blood Pb levels in the range of 1-5 µg/dL for BP and potential antecedents for CVD such as increases in TPR and changes in cardiac autonomic regulation.

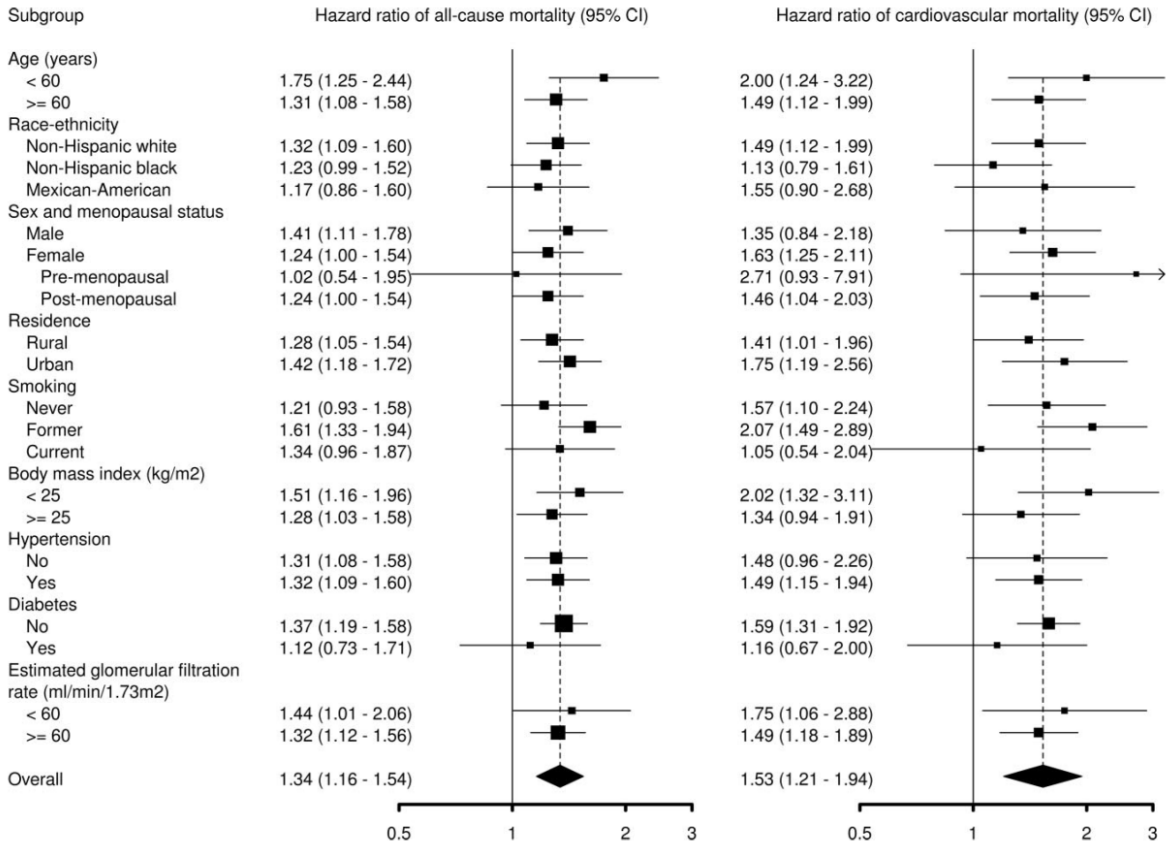
4.4.5 Mortality

The 2006 Pb AQCD (U.S. EPA, 2006b) stated that available evidence suggested an effect of Pb on cardiovascular mortality in the general U.S. population but cautioned that these findings should be replicated before these estimates for Pb-induced cardiovascular mortality could be used for quantitative risk assessment purposes (U.S. EPA, 2006b). Previous results involved NHANES II and III analyses that examined prospectively the association of adult blood Pb measured at the time of the study with all cause and cause-specific mortality ascertained 8-16 years later (Schober et al., 2006; Lustberg and Silbergeld, 2002). As blood Pb levels in adults reflect contributions from both recent Pb exposure and mobilization of historic Pb from bone, it is unclear to what extent recent, past, or cumulative Pb exposures contributed to the observed associations. Given the decline in ambient air Pb concentrations and population blood Pb levels, it is likely that study subjects had a much higher past Pb exposure compared to exposure during the study period. Using NHANES II (1976-1980) data, Lustberg and Silbergeld (2002) found significant increases in all-cause mortality, circulatory mortality, and cancer mortality, comparing adults with blood Pb levels of 20-29 µg/dL to those with blood Pb levels less than 10 µg/dL (measured 12-16 years before ascertainment of vital status). Using NHANES III data, Schober et al. (2006) found significant increased all-cause, cardiovascular, and cancer mortality comparing adults with blood Pb levels from 5-9 µg/dL and above 10 µg/dL to those with blood Pb levels less than 5 µg/dL (measured a median of 8.8 years before ascertainment of vital status).

Recent studies substantially strengthen the evidence base for Pb-associated mortality. A further analysis of the NHANES III database by a different research group using different methods addressed uncertainties from earlier analyses by considering a greater number of potential confounding factors and by characterizing concentration-response relationships. Additionally, two longitudinal prospective studies in different U.S. cohorts conducted by different researchers with different methods demonstrate consistency within the evidence base for blood Pb and add new evidence for mortality associated with bone Pb levels.

Menke et al. (2006) examined all-cause and cause-specific mortality using NHANES III data. Subjects at least 18 years of age were followed up to 12 years after their blood Pb was measured, and 1,661 deaths were identified. Those with baseline blood Pb levels from 3.63 to 10 µg/dL had significantly higher risks of all-cause (HR: 1.25 [95% CI: 1.04, 1.51]), cardiovascular (HR: 1.55 [95% CI: 1.08, 2.24]), MI (HR: 1.89 [95% CI: 1.04, 3.43]), and stroke (HR: 2.51 [95% CI: 1.20, 2.26]) mortality compared to those with baseline blood Pb levels less than 1.93 µg/dL and increased risk of cancer mortality (HR: 1.10 [95% CI: 0.82, 1.47]). Effect estimates adjusted for demographic characteristics were robust to the additional adjustment for factors such as smoking, alcohol consumption, diabetes, BMI, hypertension, and level of kidney function. The consistency of HRs across models with a varying number of control variables indicated little residual confounding. Hazard ratios were not higher comparing adults with blood Pb levels from 1.94 to 3.62 µg/dL to those with blood Pb levels <1.93 µg/dL. However, tests for linear trend were statistically significant for all mortality outcomes except for cancer mortality. Menke et al. (2006) evaluated several of the model covariates (e.g., diabetes, hypertension, and glomerular filtration rate [GFR]) in a subgroup analysis. The comparisons for these are shown in Figure 4-24. The authors reported that there were no interactions between blood Pb and other adjusted variables.

The results from Menke et al. (2006) generally were consistent with those from the previous NHANES III analysis of the association of blood Pb with mortality by Schober et al. (2006) that included participants greater than 40 years of age (N = 9,686) and adjusted for covariates including age, sex, ethnicity, and smoking rather than the full suite of covariates evaluated by Menke et al. (2006). Schober et al. (2006), which was discussed in the 2006 Pb AQCD (U.S. EPA, 2006b), reported increased HRs comparing adults with blood Pb levels ≥ 10 µg/dL to those with blood Pb levels <5 µg/dL for all-cause (HR: 1.59 [95% CI: 1.28, 1.98]), CVD (HR: 1.55 [95% CI: 1.16, 2.07]), and cancer (HR: 1.69 [95% CI: 1.14, 2.52]) mortality. In general, HRs were higher but nonsignificant, comparing adults with blood Pb levels from 5-9 µg/dL to those with blood Pb levels <5 µg/dL. The median follow-up time between measurement of blood Pb and death ascertainment was 8.55 years.



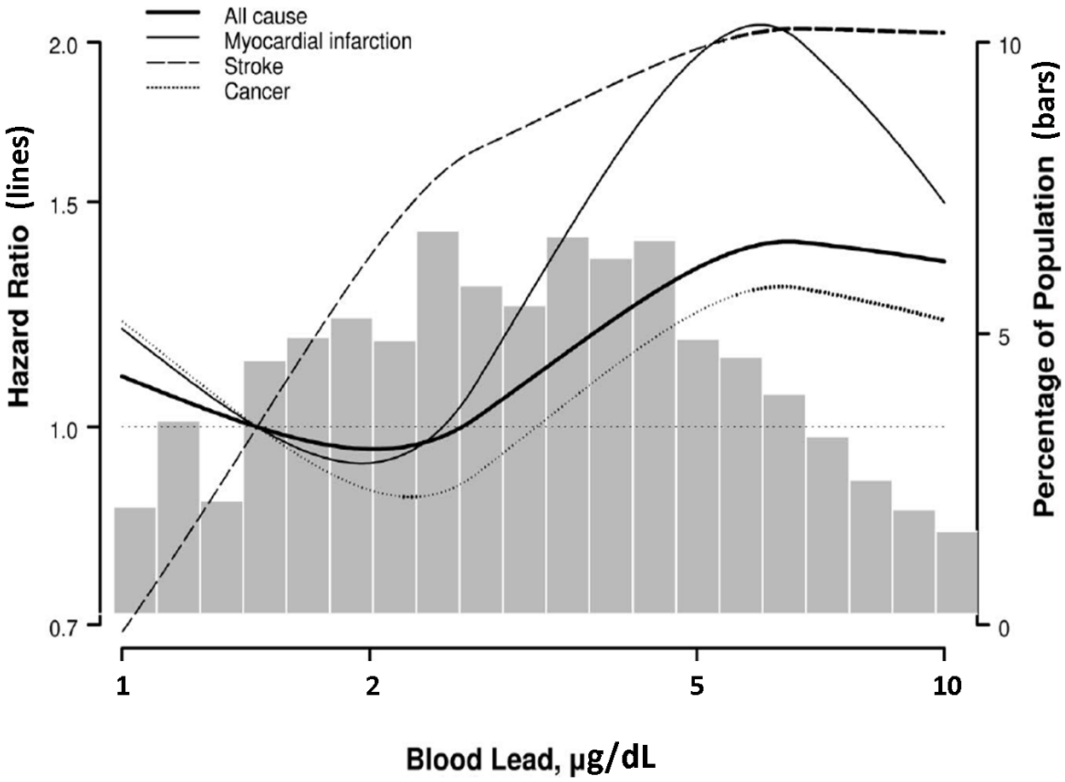
Note: Hazard ratios were calculated for a 3.4 µg/dL increase in blood Pb level with log-blood Pb as a continuous variable. This increase corresponds to the difference between the 80th and 20th percentiles of the blood Pb distribution (4.92 µg/dL versus 1.46 µg/dL, respectively).

Source: Reprinted with permission of Lippincott Williams & Wilkins, Menke et al. (2006)

Figure 4-24 Multivariate adjusted relative hazards of all-cause and cardiovascular mortality per 3.4 µg/dL increase in blood Pb.

Both Menke et al. (2006) and Schober et al. (2006) presented mortality curves that plot the HRs against blood Pb level. Figure 4-25 shows the mortality HR curves (not absolute cases of mortality) for both stroke and MI reported by Menke et al. (2006). Nonlinear associations were modeled. The curves were fitted using predetermined restricted quadratic splines with knots at the 10th percentile (1.00 µg/dL), the 50th percentile (2.67 µg/dL), and the 80th percentile (5.98 µg/dL) blood Pb levels. The authors did not explain the shape of the blood Pb-mortality curves in detail; however, the knots corresponded with the inflection points in the curve. In the tails of the blood Pb distribution, HRs decreased with increasing blood Pb level. However, HRs remained above 1 over most of the blood Pb distribution (blood Pb level greater than 2 µg/dL and between 2 and 3 µg/dL for stroke and myocardial infarction, respectively), and in the

most heavily populated portion of the blood Pb distribution, HRs increased with increasing blood Pb level. Using a referent group of persons with blood Pb level less than 1.94 µg/dL, the HR for persons with blood Pb level greater than 3.63 µg/dL was significant at the 5% level 1.51 (95% CI: 1.07, 2.14), but not significant for persons with blood Pb level in the range of 1.94 to 3.62 µg/dL. Hazard ratios peaked for all outcomes at a blood Pb level of approximately 6 µg/dL. Lower concentration-response functions at higher blood Pb levels also have been found for blood Pb-cognitive function relationships in children (Section 4.3.2).

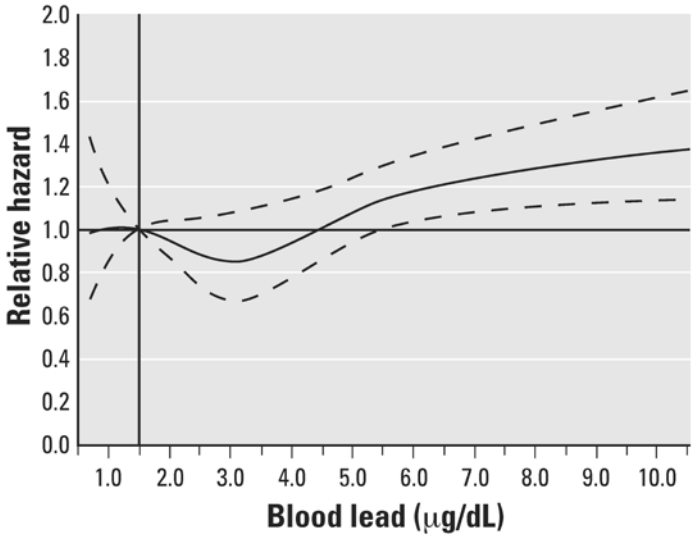


Note: A histogram of blood Pb levels is superimposed in the background and displayed on the right axis.
Source: Reprinted with permission of Lippincott Williams & Wilkins, Menke et al. (2006)

Figure 4-25 Multivariate-adjusted relative hazard (left axis) of mortality associated with blood Pb levels between 1 µg/dL and 10 µg/dL.

Schober et al. (2006) examined proportional hazard assumptions, tested for a linear trend across blood Pb tertiles, and evaluated log-transformed continuous blood Pb level as a 5-knot cubic spline (position of knots not reported). A statistically significant increasing

linear trend for mortality was observed across blood Pb tertiles. The results of the spline fit of the continuous blood Pb level term to relative hazard of all cardiovascular diseases reported by Schober et al. (2006) are shown in Figure 4-26. Schober et al. (2006) shows the upper 95% confidence band (dashed lines) of the relative risk for all cause mortality spline is greater than 1 for all blood Pb levels greater than 1.5 µg/dL using the referent group of persons with blood Pb levels less than 1.5. The HR was fixed at 1.0 for the referent blood Pb level of 1.5 µg/dL. Also, the lower 95% confidence band is greater than 1 when the blood Pb level is greater than about 4.5 µg/dL. Using a referent group of persons with blood Pb levels less than 5 µg/dL, they found statistically significant relative risks of CVD mortality for persons with blood Pb levels in the range of 5 to 9, and those with blood Pb levels greater than 10. In contrast to the curve presented by Menke et al. (2006), Schober et al. (2006) found the relative hazard axis and the blood Pb axis largely to be linear (solid line). Both Menke et al. (2006) and Schober et al. (2006) agree that persons with blood Pb levels greater than 4.5 µg/dL are at increased risk for mortality; however, these studies report different shapes for the concentration-response curves. Despite differences in the age groups included, follow-up time, categorization of blood Pb levels, and differences in HR across the blood Pb range, results reported by Menke et al. (2006) and Schober et al. (2006) find associations between higher blood Pb and increased CVD mortality (see Figure 4-29).



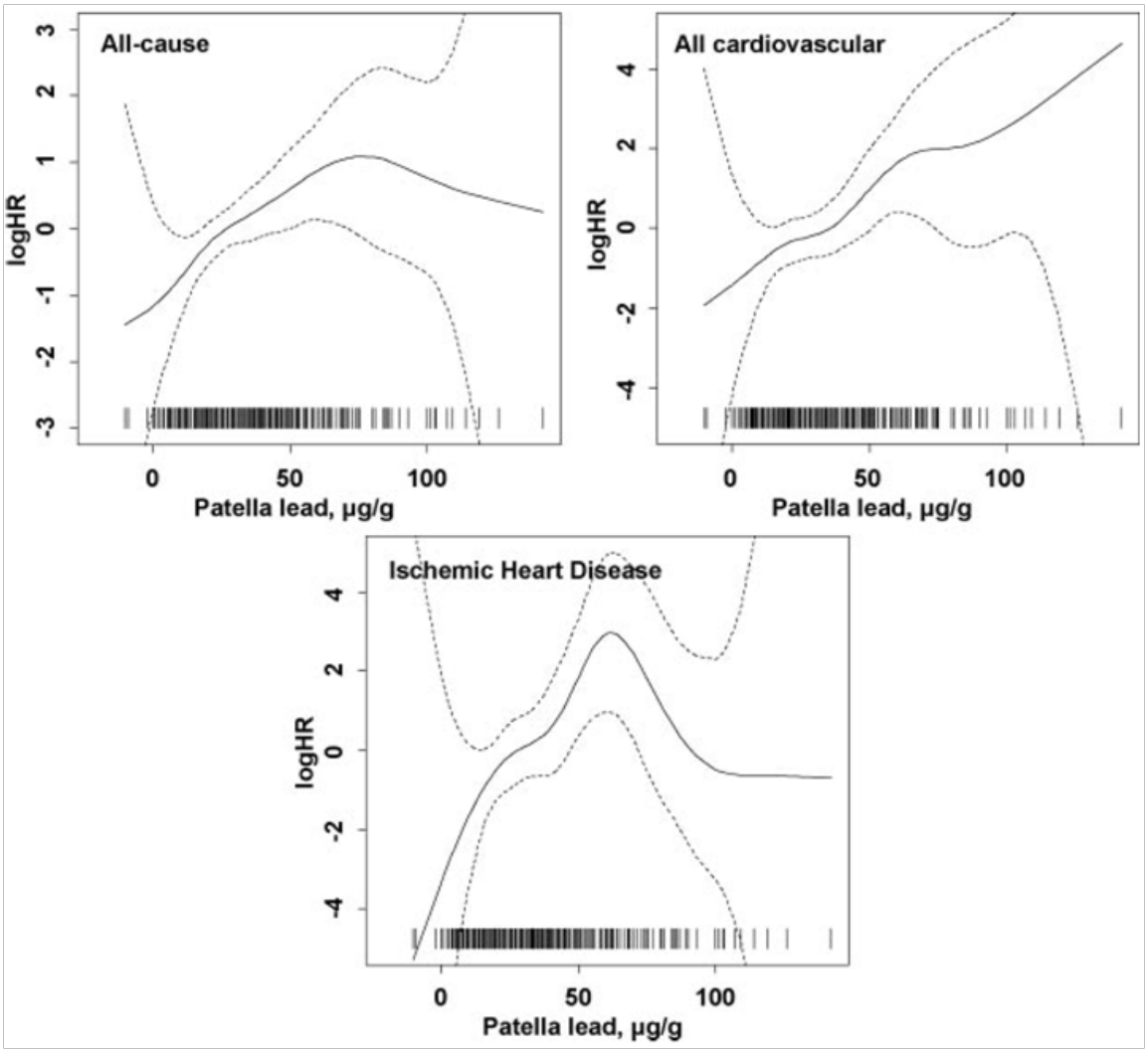
Source: Schober et al. (2006)

Note: The solid line shows the fitted five-knot spline relationship; the dashed lines are the point-wise upper and lower 95% CIs.

Figure 4-26 Relative risk of all cause mortality for different blood Pb levels compared with referent level of 1.5 µg/dL (12.5th percentile).

In addition to the NHANES analyses described above, studies of older adult, primarily white, males (Weisskopf et al., 2009) and older adult females (Khalil, 2010; Khalil et al., 2009b) were conducted recently. Weisskopf et al. (2009) used data from the NAS to determine the associations of blood, tibia, and patella Pb with mortality. The authors identified 241 deaths over an average observation period of 8.9 years (7,673 person-years). The strongest associations were observed between mortality and baseline patella Pb concentration. Baseline tibia Pb levels were more weakly associated with CVD mortality. Tibia bone Pb level is thought to reflect a longer cumulative exposure period than is patella bone Pb level because the residence time of Pb in trabecular bone is shorter than that in cortical bone. IHD contributed most to the relationship between patella Pb and all CVD deaths with an individual HR of 2.69 (95% CI: 1.42, 5.08). Although there was high correlation between tibia and patella Pb (Pearson r = 0.77), compared with cortical bone Pb, trabecular bone Pb may have more influence on circulating blood Pb level and thus, local organ concentration of Pb because of its shorter residence time in bone. In contrast to the NHANES analyses, the NAS study found that baseline blood Pb was not significantly related to cardiovascular mortality. This discrepancy may be related to differences in sample size and resulting power, modeling strategies (e.g., linear versus log-linear blood Pb level terms), or age range of the study populations. The duration of follow-up was similar across studies. In the Weisskopf et al. (2009) study of NAS data, the youngest subjects at baseline were approximately 50-55 years old, compared to the youngest in the Menke et al. (2006) and Schober et al. (2006) NHANES studies, who were 18 and 40 years, respectively. Further, the blood Pb tertile analysis of Weisskopf et al. (2009) could have been affected if the majority of a hypothesized nonlinear association was contained largely in the lowest (reference) blood Pb tertile.

Weisskopf et al. (2009) also conducted a concentration-response analysis. A linear trend was observed for increasing HR across tertiles of both tibia and patella Pb levels. The linear relationship using tertile patella Pb was confirmed in other models in which continuous patella Pb and nonlinear penalized spline terms (higher order terms) were not statistically significant. The number of knots and their placement within the Pb distribution, which can influence these results, were determined by an iterative best fit procedure. Concentration-response relationships shown in Figure 4-27 were approximately linear for patella Pb on the log HR scale for all CVD, but appeared nonlinear for IHD ($p < 0.10$). The peak HR is shown around 60 µg/g, beyond which the HR tends to decrease. It is important to note the wide confidence limits, which increase uncertainty at the lower and upper bounds of patella Pb levels.



Note: The reference logHR = 0 at the mean of patella Pb concentration. The estimates are indicated by the solid line and the 95% pointwise confidence intervals (Cis) by the dashed lines. The p values for significance of the nonlinear component for all-cause, cardiovascular, and ischemic heart disease mortality were 0.42, 0.80, and 0.10 respectively. Patella Pb concentrations of all individual participants are indicated by short vertical lines on the abscissa. Adjusted for age, education, smoking status, and pack-years of smoking among participants without ischemic heart disease at baseline.

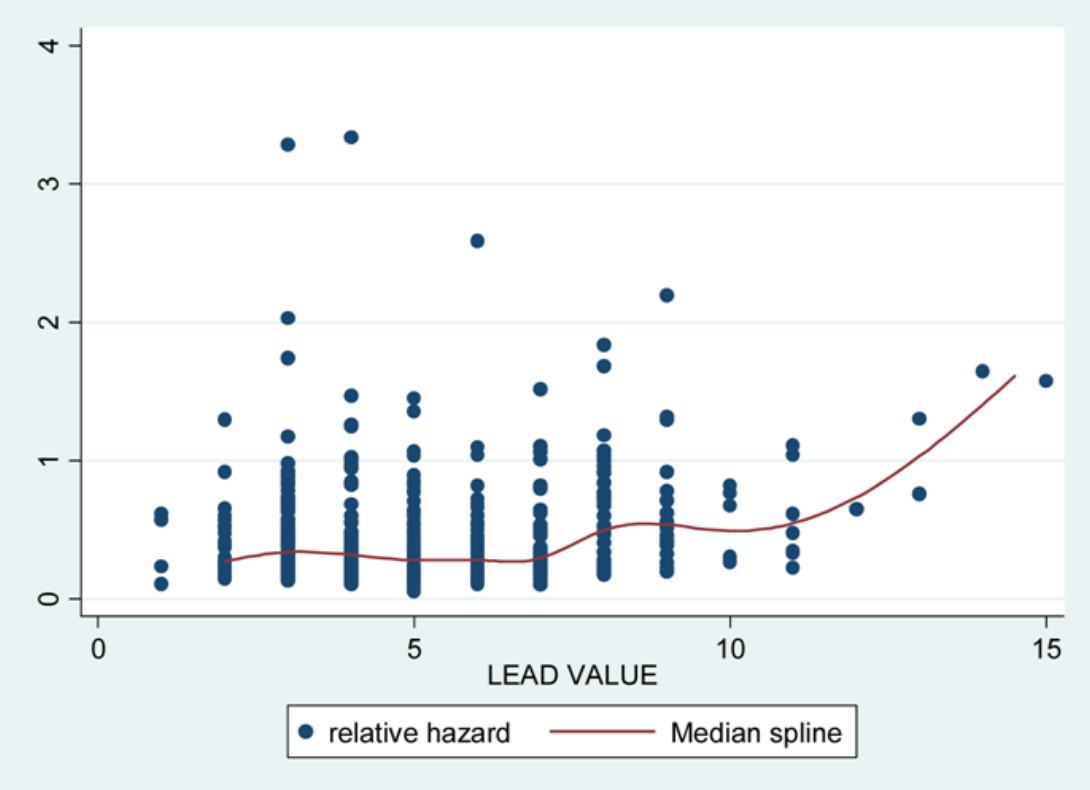
Source: Reprinted with permission of Lippincott Williams & Wilkins, Weisskopf et al. (2009)

Figure 4-27 Associations between patella bone Pb level and the log of hazard ratio (logHR) for all-cause, cardiovascular, and ischemic heart disease.

The association of adult blood Pb with mortality has also been examined among women enrolled in the Study of Osteoporotic Fractures (SOF) (Khalil et al., 2009b). This prospective cohort (N = 533) enrolled female volunteers (age 65-87 years) from two U.S. locations, Baltimore, MD and Monongahela Valley, PA and followed women for an average of 12 years after blood Pb measurement. All-cause mortality is significantly higher comparing women with blood Pb levels >8 µg/dL to those with blood Pb levels <8 µg/dL (HR: 1.59 [95% CI: 1.02, 2.49]). Hazard ratios for combined cardiovascular disease mortality (HR: 1.78 [95% CI: 0.92, 3.45]), coronary heart disease mortality (HR: 3.08 [95% CI: 1.23, 7.70]), but not stroke mortality (HR: 1.13 [95% CI: 0.34, 3.81]) were higher among the women enrolled in this study with blood Pb levels >8 µg/dL. In addition, analyses of blood Pb tertiles and quintiles indicated that blood Pb-mortality HRs were consistently elevated in groups with blood Pb levels >7 µg/dL (Khalil, 2010). The findings for elevated mortality HRs with the highest blood Pb levels are reinforced by the results displayed in Figure 4-28. The HR curve for all-cause mortality is relatively flat over most of population blood Pb distribution (represented by the blue dots) and increases only in the upper tail of the blood Pb distribution where there are relatively few subjects (i.e., fewer dots).

Other studies also reported Pb-associated increased in mortality but have limited implications due to their weaker analytic methods. Two studies reported standardized mortality ratios (SMRs) to compare observed deaths in a Pb-exposed population versus expected deaths, calculated from a reference group (Neuberger et al., 2009; Cocco et al., 2007). Mortality studies that compare populations by calculating SMRs based on an "exposed group" versus the population within which the exposed group resides have several drawbacks, including the ecologic nature of the analysis and the absence of Pb exposure data or biological markers of Pb exposure. Neuberger et al. (2009) carried out a retrospective mortality study of a Superfund site that was highly contaminated with heavy metals, principally Zn, Pb, and Cd. Not knowing the metal concentrations in the study area obscures interpretation of the significantly elevated county-state SMRs and the insignificant or significantly lowered SMRs in the county comparison.

A retrospective study of causes of death among Pb smelter workers in Sardinia, Italy followed 933 male production and maintenance workers (Cocco et al., 2007). SMRs for cardiovascular disease-related deaths were calculated based on age-specific and calendar-year specific mortality of the entire region. Significantly reduced mortality was reported in the worker groups. The authors attributed the results to the healthy worker effect based on health criteria applied at hiring and the small size of the cohort. The usual caveats regarding population comparison mortality studies apply.



Source: Khalil et al. (2010)

Figure 4-28 Multivariate adjusted relative hazard (left axis) of mortality as a function of blood Pb levels between 1 µg/dL and 15 µg/dL.

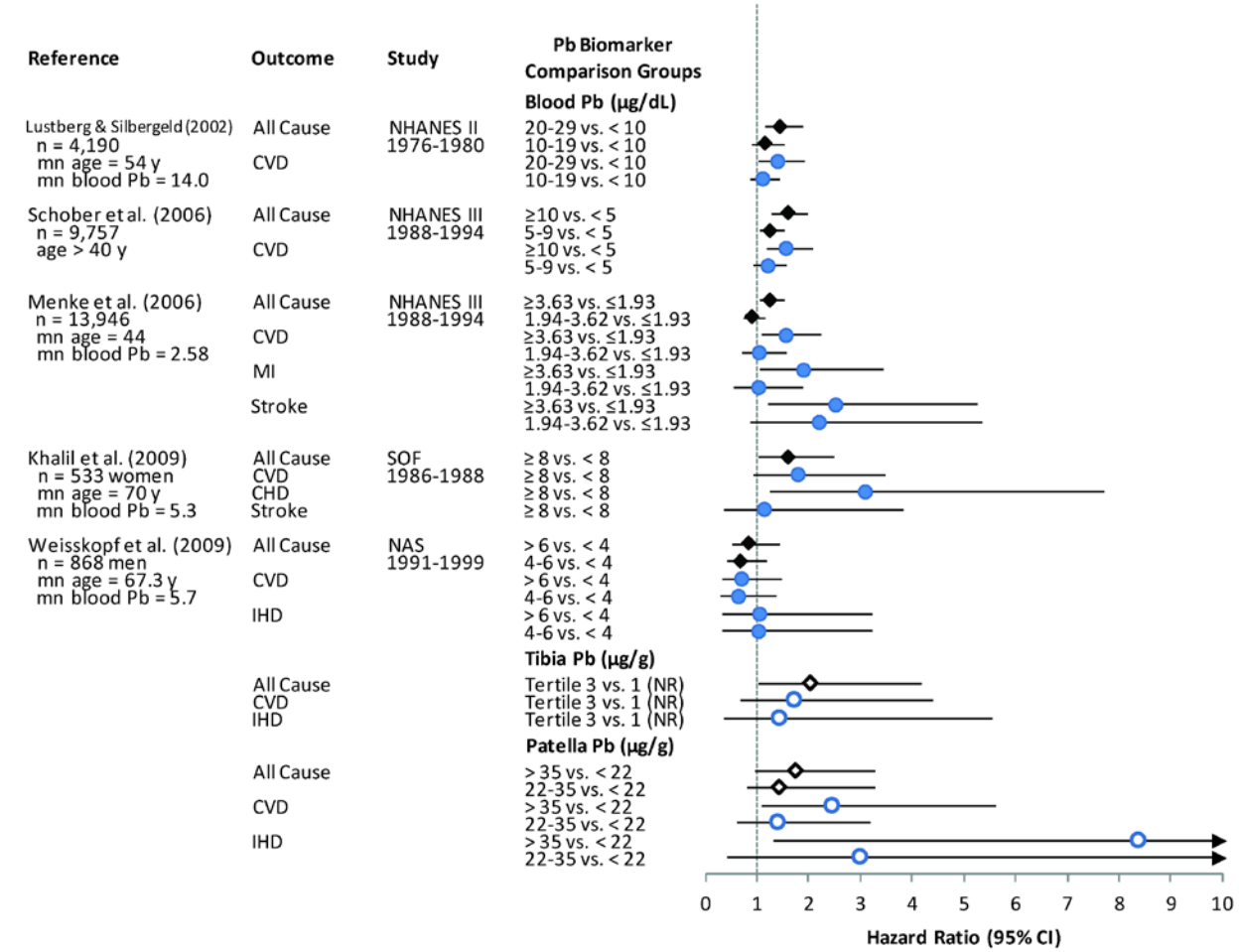
4.4.5.1 Summary of Mortality

The mortality results in this review supported and expanded upon findings from the 2006 Pb AQCD (U.S. EPA, 2006b), which included NHANES mortality studies (Schober et al., 2006; Lustberg and Silbergeld, 2002). The recent NHANES mortality study discussed above (Menke et al., 2006) had many strengths over the earlier studies, including control for a wider range of potential confounders, testing for interactions with Pb, consideration of concentration-response relationships, extensive analysis of model evaluations, and examination of mortality from specific CVDs. Further, an association with increased mortality was observed at lower mean population blood Pb levels. The mean blood Pb level of the NHANES III population was 2.58 µg/dL. In the recent analysis, the Pb risk of increased cardiovascular mortality increased with increasing blood Pb level over the most heavily populated portion of the blood Pb distribution, with

maximum blood Pb levels between 6 and 7 µg/dL. It is important to note that the relative contributions of recent, past, and cumulative Pb exposure to associations observed with the baseline blood Pb levels is uncertain. In addition, the first evidence that bone Pb, a metric of cumulative Pb exposure, is associated with increased mortality was reported recently among NAS men (Weisskopf et al., 2009).

Quantitative differences in Pb-associated hazard for death between studies may be influenced by age range of the study groups, follow up time to death, variation in model adjustment, central tendency and range of the Pb biomarker levels, assumptions of linearity in relationship with Pb biomarkers, and choice of Pb biomarker. Quantitative differences in Pb-associated mortality across NHANES II and NHANES III studies or between different NHANES III analyses may be explained by the use of continuous or ordered blood Pb terms and different data selection strategies. Further, studies using ordered categories of blood Pb level may obtain different results, as the range of blood Pb level represented in the reference category will affect the calculated coefficients of the remaining percentiles or groups.

Specifically, Menke et al. (2006) is the strongest study presently published for estimating the effects of Pb on cardiovascular disease-related mortality. The study uses the nationally representative NHANES III (1988-1994) sample of men and women. The results corroborate earlier published NHANES studies but address some of the key weaknesses noted in those studies. For example, Menke et al. (2006) examined potential confounding by a large number of factors, including hypertension and kidney function. Weisskopf et al. (2009) is the first published mortality study using bone Pb as an exposure index. The study is a prospective study with nearly 100% successful follow-up of deaths. This rigorous study found increased cardiovascular disease mortality in association with patella bone Pb with weaker associations for tibia Pb level. The Khalil et al. (2010; 2009b) study of SOF subjects provides supporting results for a cohort consisting of white females aged 65-87 years. Further, a number of prior studies found associations between accumulated Pb reflected in bone Pb measurements and higher CVD morbidity (Sections 4.4.2.1 and 4.4.3). This evidence base is augmented with new findings indicating that biomarkers of longer-term cumulative Pb exposure increases CVD mortality. The NAS and SOF examine only men and women, respectively. However, the consistency of findings between the two studies indicates that the results of either study may be applicable widely. Despite the differences in design and methods across studies, associations between higher levels of Pb bi4omarkers and higher risk of mortality were generally observed (Figure 4-29 and Table 4-23). One exception is that stroke mortality was not significantly elevated in the SOF study although it was positive. Mortality from specific CVD causes, MI and IHD mortality, which are related to higher BP and hypertension, were elevated with higher Pb biomarker levels.



Note: Studies are categorized by Pb biomarker. Within each category, studies generally are presented in order of discussion in the text. Hazard ratios represent the hazard in the higher blood or bone Pb group relative to that in the lowest blood or bone Pb group (reference group).

Blood Pb (closed markers), or Bone Pb (open markers) associations with All-cause mortality (black diamonds) or Cardiovascular mortality (blue circles).

Figure 4-29 Hazard ratios for associations of blood Pb or bone Pb with all-cause mortality and cardiovascular mortality.

Table 4-23 Additional characteristics and quantitative data for associations of blood and bone Pb with mortality for studies presented in Figure 4-29.

Study	Study Population/ Methodology	Parameter	Pb Data	Statistical Analysis	Hazard Ratio or SMR (95% CI)
Lustberg and Silbergeld (2002)	Longitudinal 4,190 adult participants of NHANES III (1976-1980), ages 30 to 74 yr, Studied through December 31, 1992	All cause and cause-specific mortality	Categorical blood Pb level Mean: 14.0 (5.1) Median: 13 µg/dL 1st tertile: <10 µg/dL (Reference) 2nd tertile: 10-19 µg/dL 3rd tertile: 20-29 µg/dL	Proportional Hazard model, RRs adjusted for age, sex, location, education, race, income, smoking, BMI, exercise	All-cause (2nd vs. 1st): 1.40 (1.16-1.69) All-cause (3rd vs. 1st): 2.02 (1.62-2.52) Circulatory (2nd vs. 1st): 1.27 (0.97, 1.57) Circulatory (3rd vs. 1st): 1.74 (1.25, 2.40) Cancer (2nd vs. 1st): 1.95 (1.28, 2.98) Cancer (3rd vs. 1st): 2.89 (1.79, 4.64)

Table 4-23 (Continued): Additional characteristics and quantitative data for associations of blood and bone Pb with mortality for studies presented in Figure 4-29.

Study	Study Population/ Methodology	Parameter	Pb Data	Statistical Analysis	Hazard Ratio or SMR (95% CI)
Schober et al. (2006)	Longitudinal 9,686 adult participants of NHANES III, ages ≥ 40 yr	All cause and cause-specific mortality	Ordered categorical blood Pb level, measured a median of 8.55 yr prior to death <5 µg/dL 5-9 µg/dL ≥ 10 µg/dL	Survey-design adjusted Cox proportional hazard adjusted for sex, age, race/ethnicity, smoking, education level. Did not evaluate BMI nor cormorbidities	All-cause (2nd vs. 1st): 1.24 (1.05, 1.48) All-cause (3rd vs. 1st): 1.59 (1.28, 1.98) CVD (2nd vs. 1st): 1.20 (0.93, 1.55) CVD (3rd vs. 1st): 1.55 (1.16, 2.07) Cancer (2nd vs. 1st): 1.44 (1.12, 1.86) Cancer (3rd vs. 1st): 1.69 (1.14, 2.52)
Menke et al. (2006)	Longitudinal 13,946 adult participants of NHANES III (1988-1994), ages ≥ 17 yr, Studied through December 31, 2000	All cause and cause-specific mortality CVD:ICD-9 390-434; ICD-10 I00-I99 MI: ICD-9 410-414 and 429.2; ICD-10 I20-I25 Stroke: ICD-9 430-434 and 436-438; ICD-10 I60-I69	Baseline blood Pb (measured an average of 12 yr before mortality): Mean: 2.58 µg/dL Tertiles: <1.93 µg/dL, 1.94-3.62 µg/dL, ≥ 3.63 µg/dL	Survey-design adjusted Cox proportional hazard regression analysis (up to 12 yr follow-up) adjusted for Model 1: age, race/ethnicity, sex, Model 2: Model 1 plus urban residence, cigarette smoking, alcohol consumption, education, physical activity, household income, menopausal status, BMI, CRP, total cholesterol, diabetes mellitus, Model 3: Model 2 plus hypertension, GFR category	All-cause (3rd vs. 1st tertile): 1.25 (1.04, 1.51) CVD (3rd vs. 1st): 1.55 (1.08, 2.24) MI (3rd vs. 1st): 1.89 (1.04, 3.43) Stroke (3rd vs. 1st): 2.51 (1.20, 5.26) Cancer (3rd vs. 1st): 1.10 (0.82, 1.47)

Table 4-23 (Continued): Additional characteristics and quantitative data for associations of blood and bone Pb with mortality for studies presented in Figure 4-29.

Study	Study Population/ Methodology	Parameter	Pb Data	Statistical Analysis	Hazard Ratio or SMR (95% CI)
Khalil et al. (2009b)	Longitudinal 533 women, ages 65-87 yr, from Study of Osteoporotic Fractures cohort in Baltimore, MD and Monongahela Valley, PA	All cause and cause-specific mortality	Blood Pb measured an average 12 (SD; 3) yr before death: Mean (SD, range): 5.3 (2.3, 1-21) µg/dL	Cox proportional hazards regression analysis adjusted for age, clinic, BMI, education, smoking, alcohol intake, estrogen use, hypertension, total hip bone mineral density, walking for exercise, and diabetes	≥ 8 µg/dL vs. <8 µg/dL All cause: 1.59 (1.02, 2.49) CVD: 1.78 (0.92, 3.45) Coronary Heart Disease: 3.08 (1.23, 7.70) Stroke: 1.13 (0.34, 3.81) Cancer: 1.64 (0.73, 3.71)

Table 4-23 (Continued): Additional characteristics and quantitative data for associations of blood and bone Pb with mortality for studies presented in Figure 4-29.

Study	Study Population/ Methodology	Parameter	Pb Data	Statistical Analysis	Hazard Ratio or SMR (95% CI)
Weisskopf et al. (2009)	Longitudinal 868 elderly men (mostly white, age >55 yr) enrolled in NAS in Greater Boston, MA area	All cause and cause-specific mortality	Pb biomarkers collected an average of 8.9 yr before death Blood Pb: Mean (SD): 5.6 (3.4) µg/dL Patella Pb: Mean (SD): 31.2 (19.4) µg/g Tertiles: <22 µg/g, 22-35 µg/g, >35 µg/g Tibia Pb: Mean (SD): 21.8 (13.6) µg/g	Cox proportional hazard regression analysis adjusted for age, smoking, education. Additional models adjusted for alcohol intake, physical activity, BMI, total cholesterol, serum HDL, diabetes mellitus, race, and hypertension	All-cause (3rd vs. 1st patella Pb tertile): 1.76 (0.95, 3.26) All CVD (3rd vs. 1st tertile): 2.45 (1.07, 5.60) IHD (3rd vs. 1st): 8.37 (1.29, 54.4) Cancer (3rd vs. 1st): 0.59 (0.21, 1.67) After excluding 154 subjects with CVD and stroke at baseline: All-cause (3rd vs. 1st): 2.52 (1.17, 5.41) All CVD (3rd vs. 1st): 5.63 (1.73, 18.3) All-cause (3rd vs. 1st blood Pb tertile): 0.93 (0.59, 1.45) All CVD (3rd vs. 1st): 0.99 (0.55, 1.78) IHD (3rd vs. 1st): 1.30 (0.54, 3.17)

Table 4-23 (Continued): Additional characteristics and quantitative data for associations of blood and bone Pb with mortality for studies presented in Figure 4-29.

Study	Study Population/ Methodology	Parameter	Pb Data	Statistical Analysis	Hazard Ratio or SMR (95% CI)
[a]Neuberger et al. (2009)	Ecological Residents at or near Tar Creek Superfund site, Ottawa County, OK (exposed pop. 5,852, unexposed pop. 16,210)	Cause-specific mortality	No biomarker measurements	Standardized mortality ratio (SMR) based on 2000 U.S. Census data	Heart disease: Both sexes: 114.1 (113.1, 115.2) Men: 118 (116.4, 119.6) Women: 111 (109.5, 112.5) Stroke: Both sexes: 121.6 (119.2, 123.9) Men: 146.7 (107.4, 195.7) Women: 106.5 (80.2, 138.6)
[a]Cocco et al. (2007)	Ecological 933 male Pb smelter workers from Sardinia, Italy (1973-2003)	All cause and cause-specific mortality	No biomarker measurements	SMR	All cause: 56 (46, 68) CVD: 37 (25, 55)

Note: Studies are generally presented in order of discussion in the text.
[a]These references not included in Figure 4-29 because they reported standardized mortality ratios.

4.4.6 Air Pb-PM Studies

4.4.6.1 Cardiovascular Morbidity

A relatively small number of studies used Pb measured in PM_{10} and $PM_{2.5}$ ambient air samples to represent Pb exposures. However, given that size distribution data for Pb-PM are fairly limited, it is difficult to assess the representativeness of these concentrations to population exposure (Section 2.5.3). Moreover, data illustrating the relationships of Pb-PM_{10} and Pb-$PM_{2.5}$ with blood Pb levels are lacking. A few available studies exposed rats, dogs, or humans to concentrated ambient air particles (CAPs) in which Pb and several other components were measured. Consistent with epidemiologic studies of blood and bone Pb and with studies of animals exposed to Pb, these studies show that exposure to Pb-containing CAPs resulted in various changes related to increased vasoconstriction (Urch et al., 2004; Wellenius et al., 2003; Batalha et al., 2002). While Pb-containing CAPs indicate cardiovascular effects with short-term exposure (2-6 hours over multiple days), they cannot be attributed specifically to the Pb component of the mixture. It is important to note that Urch et al. (2004) estimated the Pb effect on brachial artery diameter based on the ambient concentration of Pb, not direct exposure to Pb isolated from CAPs.

A U.S. time-series study of almost 3 million pregnant women found that increases in ambient Pb-TSP concentrations were associated with increased odds of pregnancy induced hypertension (PIH) assessed at delivery (4% increase per 0.05 $\mu g/m^3$ increase in seasonal average Pb at conception and birth) (Chen et al., 2006c). The authors adjusted for maternal age, race, education level, marital status, parity, and adequacy of prenatal care measured at individual levels and stratified by maternal cigarette smoking. In contrast, epidemiologic studies provide weak evidence for an association between short-term changes (daily average) in ambient air concentrations of Pb- $PM_{2.5}$ and cardiovascular morbidity in adults adjusting for weather and time trends. Some of these time-series studies analyzed Pb individually, whereas others applied source apportionment techniques to analyze Pb as part of a group of correlated components. In a time-series study of 106 U.S. counties, Bell et al. (2009) found that an increase in lag 0 Pb- $PM_{2.5}$ was associated with an increased risk of cardiovascular hospital admissions among adults ages 65 years and older. Quantitative results were not presented; however, the 95% CI was wide and included the null value. In this study, statistically significant associations were observed for other PM metal components such as nickel, vanadium, and Zn. In the absence of detailed data on correlations among components or results adjusted for copollutants, it is difficult to exclude confounding by ambient air exposures

to these other components or copollutants. To address correlations among PM chemical components, some studies applied source apportionment techniques to group components into common source categories. In these source-factor studies, it is not possible to attribute the observed association (Sarnat et al., 2008) or lack of association (Andersen et al., 2007) specifically to Pb.

4.4.6.2 Mortality

Time-series epidemiologic studies of ambient air Pb- $PM_{2.5}$ reported positive associations with mortality. Although limited in number, these studies indicated associations in multiple cities across the U.S. In the Harvard Six Cities Study, Laden et al. (2000) found a 1.16% (95% CI: 0.20, 2.9%) increased risk in all-cause mortality per 461.4 ng/m^3 (5th-95th percentile) increase in Pb-$PM_{2.5}$. In six California counties, Ostro et al. (2007) found that a 5 ng/m^3 (interquartile range) increase in Pb-$PM_{2.5}$ was associated with a 1.89% (95% CI: -0.57, 4.40%) increased risk of cardiovascular mortality and a 1.74% (95% CI: 0.24, 3.26%) increased risk of all-cause mortality during the cool season. The limitations of air-Pb studies were described in Section 4.4.6.1 above and also are relevant to the interpretation of these findings for mortality.

4.4.7 Summary and Causal Determination

Large bodies of epidemiologic and toxicological evidence indicate effects of Pb exposure on a range of related cardiovascular effects. For evaluation of causal relationships with Pb exposure, evidence was grouped in categories using the U.S. Surgeon General's Report on Smoking as a guideline (CDC, 2004). The categories include hypertension, subclinical atherosclerosis, coronary heart disease (CHD), and cerebrovascular disease. The causal determination for hypertension and increased BP is not only informed by evidence for hypertension and BP, but also cardiovascular mortality. CHD is informed by evidence in humans for HRV, MI, IHD, mortality from MI, IHD, and CHD, and in animals, increased thrombosis, coagulation, and arrhythmia. The biological plausibility and mode of action for these cardiovascular effects is provided by evidence for oxidative stress, inflammation, and vascular cell activation or dysfunction. The sections that follow describe the evaluation of evidence for these four groups of outcomes, hypertension, subclinical atherosclerosis, coronary heart disease, and cerebrovascular disease, with respect to causal relationships with Pb exposure using the framework described in Table II of the Preamble. The key evidence as it relates to the causal framework is summarized in Table 4-24.

4.4.7.1 Evidence for Hypertension and Increased Blood Pressure

The 2006 Pb AQCD concluded that there was a relationship between higher blood Pb and bone Pb and cardiovascular effects in adults, in particular increased BP and increased incidence of hypertension (U.S. EPA, 2006b), and recent evidence strengthens this conclusion. This conclusion is informed by the coherence of effects observed between epidemiologic and toxicological findings and among related endpoints. Prospective epidemiologic evidence and animal toxicology studies demonstrate the temporal relationship of the exposure to effect, while meta-analyses provide indications of consistency and strength. Cross-sectional epidemiologic evidence supports the consistency of observed results. Consideration of numerous potential confounding factors in both the prospective and cross-sectional studies limit uncertainty from bias and other lines of evidence characterizing modes of action provide biological plausibility for the associations.

Prospective studies clearly support the relationship between biomarkers of Pb exposure and hypertension incidence and BP changes establishing the directionality of effects. High-quality studies are replicated by different investigators using different designs and in large cohorts in different locations (Peters et al., 2007; Glenn et al., 2006; Cheng et al., 2001). Bone Pb coupled with high perceived stress was associated with an increased risk of developing hypertension in an originally nonhypertensive group of adults (Peters et al., 2007). Cheng et al. (2001) examined subjects from the NAS cohort without hypertension at baseline measurement and reported a significant increase for hypertension with higher patella Pb as analyzed by linear models. A recent prospective study in Pb workers found independent associations of both baseline blood Pb level and subsequent changes in blood Pb over follow-up with changes in BP over follow-up and associations of bone Pb level with hypertension (Glenn et al., 2006). The results indicated that different mechanisms may mediate short-term Pb-associated increases in BP and long-term Pb-associated development of hypertension. Consideration for key potential confounding factors was appropriate including baseline age, alcohol consumption, BMI, and use of BP lowering medications. Other factors such as smoking and education were evaluated but did not predict systolic BP. When subjects with hypertension were excluded from the model, the predicted change was not altered. Thus, the positive, statistically significant covariate-adjusted results consistently indicated in these studies help rule out chance, bias, and confounding with reasonable confidence. Figure 4-17 and Figure 4-18 and the meta-analysis indicate that results for effects of Pb exposure on BP and hypertension are positive and precise. This provides more confidence in this relationship and reduces the level of uncertainty.

The prospective evidence is supported by meta-analyses that underscore the consistency and reproducibility of Pb-associated increases in BP and hypertension across diverse populations and different study designs (Navas-Acien et al., 2008; Nawrot et al., 2002). Nawrot et al. (2002) found that each doubling of concurrent blood Pb level (between 1 and >40 μg/dL) was associated with a 1 mmHg increase in systolic BP and a 0.6 mmHg increase in diastolic BP. Navas-Acien et al. (2008) found that all included studies showed a relationship between higher bone Pb levels and higher BP. Also, all but one study that characterized hypertension showed higher relative risks or odds ratios associated with higher bone Pb levels.

Further support for a causal relationship between blood and bone Pb levels and increased BP and hypertension is provided by many cross-sectional analyses conducted by numerous researchers using different study designs and analyses in large, diverse cohorts in different locations. Evidence presented in the 1990 Pb Supplement to the Addendum (1990a) indicated that in individuals participating in NHANES II aged 40-59 years during 1976-1980, a time period corresponding with peak air Pb concentrations in the U.S., there was no evident threshold found below which blood Pb level was not significantly related to BP across a range of concurrent blood Pb level of 7-34 μg/dL. A recent study in an ethnically diverse community-based cohort of women and men aged 50-70 years found hypertension risk to be associated with blood and tibia Pb levels (Martin et al., 2006). Recent epidemiologic studies in adults found associations with hypertension in populations with relatively low mean blood Pb levels. For example, a positive relationship was found in the nationally representative NHANES III (1988-1994), in which the population geometric mean concurrent blood Pb level was 1.64 μg/dL (Muntner et al., 2005). Despite the extensive evidence for associations at relatively low concurrent blood Pb levels, these cardiovascular outcomes were most often examined in adults who have been exposed to higher levels of Pb earlier in life, and uncertainty remains concerning the Pb exposure level, timing, frequency, and duration contributing to the observed associations.

Further, recent cross-sectional epidemiologic studies also emphasized the interaction between Pb biomarker levels and factors, such as genetic variants, race/ethnicity, and metabolic syndrome, in modifying the association with BP or hypertension. Evidence was presented for a larger blood Pb-associated increase in BP in carriers of the ALAD2 allele, which is associated with greater binding affinity for Pb in the bloodstream (see Figure 4-17 for results) (Scinicariello et al., 2010). Additionally, bone Pb level was associated with larger increases in PP, which represents a good predictor of cardiovascular morbidity and mortality and an indicator of arterial stiffness, among NAS adults with the HFE H63D and/or C282Y variant (Zhang et al., 2010a) (Figure 4-17 for results). Park et al. (2009a) provided further evidence of HFE and transferrin gene

variants, related to Fe metabolism, impacting the associations of bone Pb levels with cardiovascular effects, evaluated by QT interval changes in the NAS cohort.

Combined evidence from prospective and cross-sectional studies helps limit the level of uncertainty for bias from confounding with reasonable confidence. While the adjustment for specific factors varied by study, the collective body of evidence adjusted for multiple potential key confounding factors, including age, diet, sex, BMI, blood pressure lowering medication use, SES, race/ethnicity, alcohol consumption, cholesterol, smoking, pre-existing disease (i.e., diabetes), measures of renal function, and copollutant exposures (i.e., Cd).

Associations between biomarkers of Pb exposure and increased BP and hypertension have been observed in a number of populations, including the large nationally representative NHANES cohort (Menke et al., 2006; Muntner et al., 2005). In addition, associations are found in other cohorts that include both men and women (Martin et al., 2006). Further, the meta-analyses assess cohorts both within the U.S. and international, further supporting the generalizability of the relationship between Pb exposure and increased BP and hypertension.

Cardiovascular effects of Pb exposure in children are discussed in Section 4.4.4. Overall this body of evidence, based on different cohorts, locations, and study designs is suggestive of a relationship between biomarkers of Pb exposure and cardiovascular effects in children. Recent studies provide information for BP and cardiovascular changes that may be antecedent to later-in-life effects, such as increases in TPR and changes in cardiac autonomic regulation.

A causal relationship is further supported by coherence between epidemiologic and toxicological evidence for the effects of long-term Pb exposure on BP. Collectively, all animal toxicological studies providing blood Pb level and BP measurements reported increases in BP with increasing blood Pb level in the range relevant to this ISA (Figure 4-20). Whereas the majority of studies examined long-term Pb exposures that resulted in mean blood Pb levels >10 µg/dL, one animal toxicological study found a continuous monotonic increase in BP in animals with a mean blood Pb level from 0.05 to 29 µg/dL with no evidence of a threshold (Tsao et al., 2000). Thus, most evidence demonstrated such effects in adult animals with blood Pb levels >10 µg/dL. Also, recent studies demonstrated only partial reversibility of Pb-induced increased BP following Pb exposure cessation or chelation and the possibility for short-term Pb exposure-induced increases in BP. The short-term effects were found with routes of Pb exposure that may have uncertain relevance to humans.

Coherence for BP and hypertension evidence was also provided by epidemiologic evidence indicating associations with related CV conditions. Studies in the medical literature show that increasing BP, even within the nonhypertensive range, is associated with increased rates of death and cardiovascular disease, including CHD, stroke, and cardiac failure (Ingelsson et al., 2008; Chobanian et al., 2003; Pastor-Barriuso et al., 2003; Prospective Studies Collaboration, 2002; Kannel, 2000a, b; Neaton et al., 1995). Evidence for Pb-induced hypertension and increased BP is supported by consistently observed associations between Pb biomarkers and both cardiovascular and all-cause mortality in prospective studies with follow-up periods ranging between 8 and 12 years. A recent analysis of the NHANES III sample reported associations of adult blood Pb level with cardiovascular mortality (Menke et al., 2006). These findings were supported by a community-based cohort study of women ages 65-87 years, in which higher effect estimates were observed for mortality from cardiovascular disease than all-cause mortality (Khalil et al., 2009b). Weisskopf et al. (2009) published the first mortality study using bone Pb as an exposure index. This prospective study found that patella bone Pb levels were associated with increased mortality from cardiovascular disease.

Animal toxicology studies further indicate coherence and strengthen the evidence for causality by providing strong biological plausibility for Pb-associated increases in BP and hypertension. Hypertension results from dysfunction in the regulation of blood flow and vascular resistance. Many systems, including the central and sympathetic nervous systems, the contractile processes in the vasculature, and various hormonal regulators, contribute to the maintenance of BP, and disruption of these systems will alter BP homeostasis. Studies demonstrate that oxidative stress produced following Pb exposure inactivates the vasodilator NO which may lead to increased vasoconstriction and increased BP, leading to hypertension. In addition, oxidative stress can damage the endothelium, further disrupting endothelium-dependent vascular relaxation and increasing the contractile response. Studies also suggest Pb exposure disrupts normal contractile processes by altering the sympathetic nervous system, the renin-angiotensin-aldosterone system, and the balance between production of vasodilators and vasoconstrictors (Section 4.4.2.3). Changes in BP that have been associated with biomarkers of Pb exposure indicate a modest change for an individual; however, these modest changes can have a substantial public health implication at the population level. The reported effects represent a central tendency of Pb-induced cardiovascular effects among individuals; some individuals may differ in risk and manifest effects that are greater in magnitude. For example, a small increase in BP may shift the population distribution and result in considerable increases in the percentages of individuals with BP values that are clinically significant, i.e., an indication of hypertension and medication use.

Overall, evidence in epidemiologic and toxicological studies demonstrates consistent effects of long-term Pb exposure on increased BP and hypertension in adults; however, uncertainty remains concerning the Pb exposure level, timing, frequency, and duration contributing to the effects. The epidemiologic studies are of high-quality, have been replicated by different researchers in different cohorts, and have adjusted for numerous potential confounding factors. Thus, collectively, they help limit the level of uncertainty for bias from confounding with reasonable confidence. In addition, a biologically plausible potential mode of action is described in toxicological studies. Thus, the combined evidence from epidemiologic and toxicological studies is sufficient to conclude that there is a causal relationship between Pb exposure and hypertension.

4.4.7.2 Evidence for Subclinical Atherosclerosis

Measures of subclinical atherosclerosis provide the opportunity to assess the pathogenesis of vascular disease at an earlier stage. Studies that examine markers of subclinical atherosclerosis, such as PAD (i.e., ankle-brachial index) and generalized atherosclerosis (i.e., IMT), are included in this category. A limited number of studies have evaluated markers of subclinical atherosclerosis following Pb exposure in adult humans or animals. One study described in the 2006 Pb AQCD (U.S. EPA, 2006b) indicated that Pb was associated with PAD in the NHANES population, and co-exposure with Cd did not confound the association (Navas-Acien et al., 2004). Recent epidemiologic findings are limited to cross-sectional analyses, so uncertainty exists as to the specific Pb exposure level, timing, frequency, and duration that contributed to the observed associations. One study reported an increasing trend in the odds of PAD across concurrent blood Pb level groups in adults within the NHANES population (Muntner et al., 2005), which is consistent with the results from the previous Navas-Acien et al. (2004) analysis. An occupational study presented evidence for increased measures of atherosclerosis analyzed by Doppler ultrasound (i.e., greater IMT and atherosclerotic plaque presentation) in the Pb-exposed population with a mean blood Pb level around 25 µg/dL (Poreba et al., 2011). Similarly, toxicological studies have provided limited evidence to suggest long-term Pb exposure may initiate atherosclerotic vessel disease. Pb exposure to human radial and internal mammary arteries resulted in a concentration-dependent increase in arterial intimal thickness (Zeller et al., 2010). Also, exposure to Pb in rats increased aortic medial thickness (Zhang et al., 2009a).

Toxicological studies also present evidence to clearly describe a plausible biological mechanism. Atherosclerosis is considered an inflammatory disease with a clear role for oxidative stress in the pathogenesis of the disease. There is consistent evidence that Pb exposure promotes oxidative stress and increased inflammation in animal and cell culture

models (Section 4.4.2.3). In addition, there is evidence that Pb will stimulate vascular cell activation and lead to endothelial cell dysfunction. Both events are key to the development and progression of atherosclerosis. Also, epidemiologic and animal toxicology studies have related higher blood Pb levels with higher cholesterol; high cholesterol is one of the principal risk factors for atherosclerosis (Section 4.4.3.3).

In summary, the evidence includes one high-quality epidemiologic study with adjustment for numerous potential confounders (Muntner et al., 2005) and biological plausibility for observations in humans provided by a few toxicological studies. Thus, the limited combined epidemiologic and toxicological evidence for is suggestive of a causal relationship between Pb exposure and subclinical atherosclerosis.

4.4.7.3 Evidence for Coronary Heart Disease

Coronary heart disease (CHD) results from interruption of the blood supply to a part of the heart caused by atherosclerosis of the coronary arteries, with acute injury and scarring leading to permanent damage to the heart muscles. A disrupted HRV has been associated with a higher mortality after MI and is used as a predictor of the physiological processes underlying CHD (Buccelletti et al., 2009). Studies that examine incidence of MI, IHD, HRV, and mortality from CHD, MI, or IHD are included in this category.

There were a small number of studies discussed in the 2006 Pb AQCD (U.S. EPA, 2006b) that indicated associations between Pb biomarker levels and increased risk of cardiovascular outcomes associated with CHD. However, recent longitudinal studies in cohorts in different locations with follow-up periods ranging between 8 and 12 years report that biomarkers of Pb exposure are associated with risk of mortality from cardiovascular disease, specifically MI, IHD, or CHD. A recent analysis of the NHANES III sample reported associations of adult blood Pb level with cardiovascular mortality, with stronger associations observed with MI mortality (Menke et al., 2006). These findings were supported by a community-based cohort study of women ages 65-87 years, in which higher effect estimates were observed for mortality from CHD than all-cause mortality (Khalil et al., 2009b). Weisskopf et al. (2009) published the first mortality study using bone Pb as an exposure index. This prospective study found that patella bone Pb levels were associated with increased mortality from IHD. Despite the differences in design and methods across studies, with few exceptions associations between higher levels of Pb biomarkers and higher risk of CHD-related mortality were consistently observed (Figure 4-29 and Table 4-23).

The body of evidence demonstrating associations with mortality from CHD is substantiated by several findings indicating associations between biomarkers of Pb and

incidence of CHD-related outcomes. A prospective analysis examined the incidence of IHD (physician confirmed MI, angina pectoris) in the NAS cohort and reported findings indicating that both blood and bone Pb levels contribute independently to IHD incidence (Jain et al., 2007). Earlier studies reported associations of increased Pb biomarkers with increased risk of left ventricular hypertrophy (Schwartz, 1991). Coherence for the associations in humans is provided by a recent animal study that suggested that Pb exposure promotes a procoagulant state that could contribute to thrombus formation which could reduce the blood supply to the heart (Shin et al., 2007).

Further support for a relationship between Pb exposure and CHD is provided by evidence from the NAS cohort for effects on disrupted HRV (Eum et al., 2011; Park et al., 2009a; Park et al., 2006), which has been associated with a higher mortality from MI and is used as a predictor of the physiological processes underlying CHD. A prospective analysis reported that higher tibia Pb, but not blood or patella Pb, was associated with increases in QTc interval and QRSc duration (Eum et al., 2011). Park et al. (2006) reported associations of bone Pb with HRV measures and effect modification by increasing number of Fe metabolism gene variants from 0 to 3. Park et al. (2006) reported the strongest relationships between patella Pb levels and decreased HRV among adults with three or more metabolic abnormalities.

As CHD is the result of vascular blockage, the suggestive evidence for subclinical atherosclerosis supports the observations of increased CHD morbidity and mortality with increased Pb exposure. In addition, the strong and consistent evidence for Pb-induced hypertension serves as further biological plausibility for effects on CHD. Hypertension may contribute to CHD development in a number of ways. Hypertension may lead to thickening of the vascular wall or exacerbation of atherosclerotic plaque development and thus contribute to plaque instability. In addition, hypertension may increase the myocardial oxygen demand priming for potential myocardial ischemia (Olafiranye et al., 2011). Evidence for both subclinical atherosclerosis and hypertension is supported by consistent evidence describing the mode of action including Pb-induced oxidative stress, inflammation, cellular activation and dysfunction, altered vascular reactivity, RAAS dysfunction, and vasomodulator imbalance.

Recent epidemiologic and toxicological studies substantiated the evidence that long-term Pb exposure is associated with CHD in adults; however, uncertainty remains concerning the Pb exposure level, timing, frequency, and duration contributing to the effects. Overall, high-quality studies examining CHD morbidity and mortality and contributing cardiovascular effects have been replicated by different researchers in different cohorts and report consistent associations that increase the confidence that a relationship exists between Pb exposure and CHD. In addition, both animal and human studies describe a

biologically plausible potential mode of action. Thus, the overall evidence from primarily epidemiologic studies and studies providing plausible mode of action information is sufficient to conclude that there is a causal relationship between Pb exposure and coronary heart disease.

4.4.7.4 Evidence for Cerebrovascular Disease

Cerebrovascular disease describes a group of conditions involving the cerebral blood vessels that result in transient or permanent disruption of blood flow to the brain. These conditions include stroke, transient ischemic attack, and subarachnoid hemorrhage. Both hypertension and atherosclerosis are risk factors for cerebrovascular disease and the mechanisms for these outcomes also apply to cerebrovascular disease. Despite strong evidence for hypertension and CHD and long-term Pb exposure, very few studies have examined the effects of Pb exposure on cerebrovascular disease. Lee et al. (2009) examined 153 patients in Taiwan cross-sectionally while adjusting for key potential confounders and reported increased stenosis greater than 50% in the intracarotid system related to urine Pb but not blood Pb level. Two epidemiologic studies prospectively evaluated mortality from stroke. In the NHANES study, Menke et al. (2006) reported a positive relationship between blood Pb levels and stroke mortality but with wider confidence intervals compared to other outcomes examined. Khalil et al. (2009b) reported a nonsignificant result with imprecise confidence intervals. These few studies provide insufficient evidence to inform the causal relationship between cerebrovascular disease and long-term Pb exposure. Thus, the evidence at this time is inadequate to determine that a causal relationship exists between Pb exposure and cerebrovascular disease.

Table 4-24 Summary of evidence supporting cardiovascular causal determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Exposure or Biomarker Levels Associated with Effects[c]
Hypertension – Causal			
Consistent associations from multiple, high quality epidemiologic studies with relevant blood Pb levels	Longitudinal evidence for associations with incidence of hypertension and increase in blood pressure in adults.	Peters et al. (2007), Glenn et al. (2006), Cheng et al. (2001)	Adult, prospective[d]: Blood Pb level >20 µg/dL; Bone Pb level >20 µg/g
	Large body of supportive cross-sectional studies applying differing designs across multiple cohorts of adults in different locations.	Martin et al. (2006), Scinicariello et al. (2010), Park et al. (2009b)	Adult, concurrent: Blood Pb level >2 µg/dL; Bone Pb level >19 µg/g
Additional epidemiologic evidence help rule out chance, bias, and confounding with reasonable confidence	Associations found with adjustment for numerous potential confounding factors including age, sex, diet, BMI, SES, race/ethnicity, alcohol, cholesterol, smoking, medication use, pre-existing disease, renal function, and copollutants exposures.	Section 4.4.2.1	
	Studies had population-based recruitment, most with moderate to high follow-up participation not conditional on blood or bone Pb level.	Table 4-18, Table 4-19	
	Meta-analyses demonstrate consistency of association	Navas-Acien et al. (2008), Nawrot et al. (2002)	
Consistent evidence from well conducted animal studies at relevant exposures help rule out chance, bias, and confounding with reasonable confidence	Well controlled studies in adult rodents with relevant dietary long-term Pb exposure support the consistent evidence for increases in BP in adults.	Rodents: Rizzi et al.(2009), Bravo et al.(2007), Chang et al.(2005), Tsao et al. (2000) Section 4.4.2.2	Rat, adult: Blood Pb level >10 µg/dL

Table 4-24 (Continued): Summary of evidence supporting cardiovascular causal determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Exposure or Biomarker Levels Associated with Effects[c]
Consistent associations from multiple, high- quality epidemiologic studies with relevant Pb levels in blood and/or bone and cardiovascular mortality	Longitudinal studies find consistent associations of blood and/or bone Pb levels in adults with risk of cardiovascular mortality applying differing designs across multiple cohorts in different locations	Khalil et al. (2009b), Weisskopf et al. (2009), Menke et al. (2006) Schober et al. (2006) Lustberg and Silbergeld (2002)	Adult, prospective: Blood Pb level >4 µg/dL
Additional epidemiologic evidence to rule out chance, bias, and confounding with reasonable confidence	Associations found with adjustment for numerous potential confounding factors. Studies had population-based recruitment not conditional on blood or bone Pb level.	Section 4.4.5	
Evidence clearly describes mode of action			
Oxidative Stress	Consistent evidence of increased oxidative stress leading to inactivation of NO and downregulation of sGC in animals with relevant dietary Pb exposures and cultured vascular cells.		
Alteration of vascular reactivity	Toxicological evidence for activation of the sympathetic nervous system, increased reactivity to catecholamines, and activation of the adrenergic and dopaminergic receptors in rats, isolated vessels, and cultured cells. Mixed evidence for reactivity to other pressor agents (e.g., 5-HT) in rats.	Section 4.4.2.3	
Renin-angiotensin-aldosterone system dysfunction	Toxicological evidence that activation of the RAAS may be involved in development of Pb-induced hypertension Evidence for increased RAAS activity in rats and decreased BP following RAAS inhibition and Pb exposure.		
Vasomodulator imbalance	Limited available toxicological evidence reporting vasomodulator imbalance in Pb-exposed rats and cells.		

Table 4-24 (Continued): Summary of evidence supporting cardiovascular causal determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Exposure or Biomarker Levels Associated with Effects[c]
Subclinical Atherosclerosis – Suggestive			
Limited but high-quality evidence in humans of an association with subclinical atherosclerosis and peripheral artery disease	One NHANES analysis reported associations with PAD at relevant adult blood Pb levels with adjustment for numerous potential confounding factors.	Muntner et al. (2005)	Adult, concurrent: Blood Pb level >2.5 µg/dL
	Limited evidence for increased IMT or arterial stiffness in adult human populations.	Ari et al. (2011)	Adult, concurrent: Serum Pb level >0.4 µg/dL
	Occupational studies report increased IMT and atherosclerotic plaque presentation in highly exposed adult populations.	Poręba et al. (2011; 2011a) Sections 4.4.3.3 and 4.4.3.5	Adult workers: Blood Pb level >24 µg/dL
Limited evidence in animals of initiation or progression of atherosclerosis after Pb exposure	A few studies reported increased IMT, vascular morphological changes, and endothelial and SMC alterations in rats and human tissue.	Zeller et al. (2010), Zhang et al. (2009a) Section 4.4.3.3	Rat: 28.4 µg/dL Human Tissue: 50 µM
Evidence clearly describes mode of action			
Oxidative Stress	Consistent evidence of increased oxidative stress in animals with relevant dietary Pb exposures and cultured vascular cells.	Section 4.4.2.3	
Inflammation	Toxicological evidence of increased inflammation as indicated by increased production of TNF-α, IL-6, IL-8, and PGE_2 by macrophages and vascular cells.		
Vascular Cell Activation and Endothelial Dysfunction	Toxicological evidence of VSMC stimulation and endothelial dysfunction and damage in culture. Limited available evidence of impaired flow-mediated dilatation in Pb exposed workers.	Section 4.4.3.1	

Table 4-24 (Continued): Summary of evidence supporting cardiovascular causal determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Exposure or Biomarker Levels Associated with Effects[c]
Coronary Heart Disease – Causal			
Consistent associations from multiple, high quality epidemiologic studies with relevant bone and/or blood Pb levels and mortality from MI, IHD, CHD, and cardiovascular disease	Longitudinal studies find consistent associations of bone and/or blood Pb levels in adults with risk of cause-specific cardiovascular mortality applying differing designs across multiple cohorts in different locations.	Khalil et al. (2009b), Weisskopf et al. (2009), Menke et al. (2006) Schober et al. (2006) Lustberg and Silbergeld (2002)	Adult, prospective: Blood Pb level >4 µg/dL
Limited but supporting evidence in humans of an association with IHD, MI, or HRV	One prospective study demonstrates an association of adult blood and bone Pb levels with incidence of IHD in the NAS cohort	Jain et al. (2007)	Adult, prospective: Blood Pb level >5 µg/dL
	Associations of Pb levels in adults and left ventricular hypertrophy and MI	Schwartz (1991)	
	Prospective evidence of association of HRV with tibia bone Pb level in adults.	Eum et al. (2011)	Adult, prospective: Bone Pb level >23 µg/g
	Evidence for interaction of markers of metabolic syndrome and genetic polymorphisms with Pb-induced HRV.	Park et al. (2009a; 2006)	
		Sections 4.4.3.4 and 4.4.3.6	
Additional epidemiologic evidence help rule out chance, bias, and confounding with reasonable confidence	Associations found while adjusting for numerous potential confounding factors.	Section 4.4.5	
	Studies had population-based recruitment not conditional on blood or bone Pb level.		
Limited but supporting evidence in animals of increased thrombosis, enhanced coagulation, and arrhythmia	One study reporting increased thrombosis and enhanced coagulation in rats and cells.	Shin et al. (2007)	Rat Blood Pb level: 26.8 µg/dL
	One study reporting increased incidence of arrhythmia and atrioventricular conduction block in rats.	Reza et al. (2008)	
		Sections 4.4.3.4 and 4.4.3.6	

Table 4-24 (Continued): Summary of evidence supporting cardiovascular causal determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Exposure or Biomarker Levels Associated with Effects[c]
Evidence clearly describes mode of action			
Oxidative Stress	Consistent evidence of increased oxidative stress in animals with relevant dietary Pb exposures and cultured vascular cells.	Section 4.4.2.3	
Inflammation	Toxicological evidence of increased inflammation as indicated by increased production of TNF-α, IL-6, IL-8, and PGE_2 by macrophages and vascular cells.	Section 4.4.3.1	
Atherosclerosis	Suggestive evidence of subclinical atherosclerosis in humans and animals with relevant Pb exposure resulting in narrowing of the blood vessels to the heart.	Sections 4.4.3.3 and 4.4.3.5	
Hypertension	Consistent evidence of increased BP and hypertension following Pb exposure in humans and animals at relevant Pb levels across numerous studies with control for confounding. Association of increased blood pressure with manifestation of CHD has been well documented.	Section 4.4.2	
Cerebrovascular Disease - Inadequate			
Evidence for cerebrovascular disease in humans and animals is of insufficient quality and quantity	One study reported an association of intracranial carotid stenosis with urinary Pb level in adults.	Lee et al. (2009) Section 4.4.3.3	Adult, concurrent: Blood Pb level >5 µg/dL
Limited evidence for increased mortality from stroke	Limited evidence for increased risk of mortality from stroke across two cohorts in different locations.	Menke et al. (2006), Khalil et al. (2009b) Section 4.4.5	Adult, prospective: Blood Pb level >4 µg/dL

Table 4-24 (Continued): Summary of evidence supporting cardiovascular causal determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Exposure or Biomarker Levels Associated with Effects[c]
Evidence for possible mode of action			
Hypertension	Consistent evidence of increased BP and hypertension following Pb exposure in humans and animals at relevant Pb levels across numerous studies with adjustment for numerous potential confounding factors. Association of increased blood pressure with manifestation of CHD has been well documented.	Section 4.4.2	
Atherosclerosis	Suggestive evidence of subclinical atherosclerosis in humans and animals with relevant Pb exposure resulting in narrowing of the blood vessels to the heart.	Sections 4.4.3.3 and 4.4.3.5	

[a]Described in detail in Table II of the Preamble.

[b]Describes the key evidence and references contributing most heavily to causal determination and where applicable to uncertainties and inconsistencies. References to earlier sections indicate where full body of evidence is described.

[c]Describes the blood or bone Pb levels in humans with which the evidence is substantiated and blood Pb levels in animals most relevant to this ISA.

[d]Because blood Pb level in nonoccupationally-exposed adults reflects both recent and past Pb exposures, the magnitude, timing, frequency, and duration of Pb exposure contributing to the observed associations is uncertain.

4.5 Renal Effects

4.5.1 Introduction

This section summarizes key findings with regard to effects of Pb on the kidney in animal toxicology and epidemiologic studies. Toxicological studies report that long-term Pb exposure is associated with pathological changes in the renal system such as proximal tubule (PT) cytomegaly, renal cell apoptosis, mitochondrial dysfunction, aminoaciduria, increased electrolyte excretion, ATPase dysfunction, oxidant redox imbalance, and altered NO homeostasis with ensuing elevated BP. Epidemiologic studies provide evidence for altered kidney function and chronic kidney disease (CKD) development. The cardiovascular and renal systems are intimately linked. Homeostatic control at the kidney level functions to regulate water and electrolyte balance via filtration, re-absorption and excretion and is under tight hormonal control. Pb exposure has been shown to damage the kidneys and its vasculature with ensuing effects on systemic hypertension and effects on the cardiovascular (Section 4.4) and renal systems. Chronic increases in vascular pressure can contribute to glomerular and renal vasculature injury, which can lead to progressive renal dysfunction and kidney failure. In this manner, Pb-induced hypertension has been regarded as one potential contributor of Pb-induced renal disease. However, the relationship between BP and renal function is more complicated. Not only does hypertension contribute to renal dysfunction but damage to the kidneys can also cause increased BP. Long-term control of arterial pressure is affected by body fluid homeostasis which is regulated by the kidneys. In examination of the physiological definition of BP (i.e., mean BP equates to cardiac output multiplied by total peripheral resistance [TPR]) the role of the kidneys in BP regulation is highlighted. Cardiac output is driven by left ventricular and circulating blood volume. TPR is driven by vasomodulation and electrolyte balance. Thus, it is possible to dissect the causes of hypertension from features of primary kidney disease. Increased extracellular fluid volume results in increased blood volume which enhances venous return of blood to the heart and increases cardiac output. Increased cardiac output not only directly increases BP, but also increases TPR due to a compensatory autoregulation or vessel constriction. In addition, damage to the renal vasculature will alter the intra-renal vascular resistance thereby altering kidney function and affecting the balance between renal function and BP. The interactions between these systems can lead to further exacerbation of vascular and kidney dysfunction following Pb exposure. As kidney dysfunction can increase BP and increased BP can lead to further damage to the kidneys, Pb-induced damage to both systems may result in a cycle of further increased severity of disease.

In general, associations between bone Pb (particularly in the tibia) and health outcomes in adults indicate chronic effects of cumulative Pb exposure. In adults without current occupational Pb exposure, blood Pb level represents both recent and cumulative Pb exposure. In particular, blood Pb level may represent cumulative exposure in physiological circumstances of increased bone remodeling or loss (e.g., osteoporosis and pregnancy) when Pb from bone of adults contributes substantially to blood Pb concentrations. Blood Pb level in children is also influenced by Pb stored in bone due to rapid growth-related bone turnover in children relative to adults. Thus, blood Pb in children is also reflective of cumulative dose. Additional details on the interpretation of Pb in blood and bone are provided in Section 3.3.5. The toxicokinetics of Pb in blood and bone are important considerations in making inferences about etiologically-relevant Pb exposures that contributed to associations observed between blood and bone Pb levels and health outcomes.

4.5.1.1 Kidney Outcome Measures

The primary function of the kidneys is to filter waste from the body while maintaining appropriate levels of water and essential chemicals, such as electrolytes, in the body. Therefore, the gold standard for kidney function assessment involves measurement of the glomerular filtration rate (GFR) through administration of an exogenous radionuclide or radiocontrast marker (e.g., 125I-iothalamate, iohexol) followed by timed sequential blood samples or, more recently, kidney imaging, to assess clearance through the kidneys. This procedure is invasive and time-consuming. Therefore, serum levels of endogenous compounds are routinely used to estimate GFR in large epidemiologic studies and clinical settings. Creatinine is the most commonly measured endogenous compound; blood urea nitrogen (BUN) has also been examined. Increased serum concentration or decreased kidney clearance of these markers both indicate decreased kidney function. The main limitation of endogenous compounds identified to date is that non-kidney factors impact their serum levels. Specifically, since creatinine is derived from creatine in muscle, muscle mass and diet affect serum levels resulting in variation in different population subgroups (e.g., women and children compared to men), that are unrelated to kidney function. Measured creatinine clearance, involving measurement and comparison of creatinine in both serum and urine, can address this problem. However, measured creatinine clearance utilizes timed urine collections, traditionally over a 24-hour period, and the challenge of complete urine collection over an extended time period makes compliance difficult.

Therefore equations to estimate kidney filtration that utilize serum creatinine but also incorporate age, sex, race, and, in some, weight (in an attempt to adjust for differences in

muscle mass), have been developed. Although these are imperfect surrogates for muscle mass, such equations are currently the preferred outcome assessment method. Traditionally, the Cockcroft-Gault equation (Cockcroft and Gault, 1976), which estimates creatinine clearance, a GFR surrogate, has been used. In the last decade, the abbreviated Modification of Diet in Kidney Disease (MDRD) Study equation (Levey et al., 2000; Levey et al., 1999), which estimates GFR, has become the standard in the kidney epidemiologic and clinical communities. With widespread use of the MDRD equation, it became clear that the equation underestimates GFR at levels in the normal range. Therefore, the CKD-Epidemiology Collaboration (CKD-EPI) equation was recently developed to be more accurate in this range (Levey et al., 2009). This is a decided advantage in nephrotoxicant research since most participants in occupational and many even in general population studies have GFRs in a range that is underestimated by the MDRD equation.

Both the MDRD and CKD-EPI equations use serum creatinine. Due to the inability to adjust serum creatinine levels for muscle mass, alternative serum biomarkers have been evaluated such as cystatin C, a cysteine protease inhibitor that is filtered, reabsorbed, and catabolized in the kidney (Fried, 2009). It is produced and secreted by all nucleated cells thus avoiding the muscle mass confounding that exists with serum creatinine (Fried, 2009). However, recent research indicates that serum cystatin C varies by age, sex, and race (Kottgen et al., 2008). Thus, a cystatin C-based eGFR equation was recently developed that includes age, sex, and race (Stevens et al., 2008).

Most of the kidney outcome measures discussed above were developed for use in the clinical setting, but are insensitive for detection of early kidney damage. This lack of sensitivity is evidenced by the fact that serum creatinine remains normal after kidney donation. Therefore, in the last two decades, the utility of early biological effect (EBE) markers as indicators of preclinical kidney damage has been of interest. These can be categorized as markers of function (i.e., low molecular weight proteins that should be reabsorbed in the PT such as β2-microglobulin and retinol-binding protein [RBP]); biochemical alteration (i.e., urinary eicosanoids such as prostaglandin E2, prostaglandin F2 alpha, 6-keto-prostaglandin F_1 alpha, and thromboxane B2); and cytotoxicity (e.g., N-acetyl-β-D-glucosaminidase [NAG]) (Cardenas et al., 1993). Elevated levels may indicate an increased risk for subsequent kidney dysfunction. However, most of these markers are research tools only, and their prognostic value remains uncertain since prospective studies of most of these markers in nephrotoxicant-exposed populations are quite limited to date. Recently, microalbuminuria has been identified as a PT marker, not just glomerular as previously thought (Comper and Russo, 2009). Kidney EBE markers are a major recent focus for research in patients with acute kidney injury (AKI) and markers such as neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury

molecule-1 (Kim-1), developed in AKI research, may prove useful for chronic nephrotoxicant work as well (Ferguson et al., 2008; Devarajan, 2007).

4.5.2 Nephrotoxicity and Renal Pathology

A number of advances in the impact of Pb on the kidney in the 20 years following the 1986 Pb AQCD (U.S. EPA, 1986a) were noted in the 2006 Pb AQCD (U.S. EPA, 2006b). These advances included evidence that Pb is associated with kidney dysfunction in both the general population and among CKD patients with mean blood Pb levels from 5-10 µg/dL. Furthermore Pb exposure, at much lower doses than those causing chronic Pb nephropathy, may act as a cofactor with other more established kidney risks to increase the risk of CKD and disease progression in susceptible patients. Maric and Hall (2011) note that data from basic and clinical studies suggest that obesity, hypertension, hyperglycemia, hyperlipedemia, and other elements of the metabolic syndrome are highly interrelated and contribute to the development and progression of diabetic nephropathy and thus represent populations potentially at increased risk for kidney dysfunction.

Several key uncertainties in the relationship between Pb exposure and kidney function were noted in the 2006 Pb AQCD (U.S. EPA, 2006b). First, because the effects on the kidney are most often observed in adults with likely higher past Pb exposures, uncertainty exists as to the Pb exposure level, timing, frequency, and duration contributing to the associations observed with blood or bone Pb levels. In addition, there are uncertainties relating to the impacts of Pb on the kidney in children, the use of paradoxical Pb-kidney associations on risk assessment in the occupational setting, and the impact of co-exposure to other environmental nephrotoxicants, such as Cd.

Another important uncertainty relates to the potential for reverse causality, i.e. the potential for reduced excretion of Pb associated with diminished kidney function rather than a causal association, to explain relationships observed between blood Pb level and kidney function. Reverse causality is most important for the interpretation of cross-sectional studies where the temporal relationship between Pb exposure and kidney function is not well-established. Although reverse causality has been typically considered plausible in the interpretation of studies of CKD patients whose kidneys are known to be compromised, the reverse causality hypothesis can be broadened to include a physiologic process in which Pb levels are influenced over the entire range of kidney filtration rates. This expanded hypothesis implies that low blood and bone Pb levels may reflect kidney function in addition to exposure. If so, this would increase misclassification bias, with Pb biomarkers reflecting both exposure and kidney function. Various qualitative reviews and editorials discuss the potential for reverse causality and reach different conclusions

(Bellinger, 2011; Evans and Elinder, 2011; Kosnett et al., 2007; Marsden, 2003). This uncertainty will be discussed in greater detail in Section 4.5.2.5.

4.5.2.1 Epidemiology in Adults

General Population Studies

The 2006 Pb AQCD (U.S. EPA, 2006b) included studies that examined associations between indicators of Pb exposure and kidney function in the general population. The body of evidence available in adults is summarized in Figure 4-30 and Table 4-25 and discussed next. Although positive statistically significant associations were not observed in each of the studies, collectively the findings represent a pattern of elevated risks for reduced kidney function following long-term Pb exposure.

Two longitudinal analyses of the normative aging study (NAS) population report associations between blood Pb and increased serum creatinine (Tsaih et al., 2004; Kim et al., 1996). These analyses reduce uncertainties related to reverse causality because they establish the temporal relationship between Pb exposure and kidney dysfunction. Participants in NAS, who were originally recruited in the 1960s in the Greater Boston area were male, age 21 to 80 years, and without chronic medical conditions. The first of these studies included 459 men whose blood Pb levels from periodic examinations, conducted every 3 to 5 years during 1979-1994, were estimated based on measurements in stored packed red blood cell samples adjusted for hematocrit level (Kim et al., 1996). Participants were randomly selected to be representative of the entire NAS population in terms of age and follow-up. Kidney function was assessed with serum creatinine. Data from four evaluations were available for the majority of participants. Mean (SD) age, blood Pb level, and serum creatinine, at baseline, were 56.9 (8.3) years, 9.9 (6.1) μg/dL, and 1.22 (0.2) mg/dL, respectively. In the longitudinal analysis with random-effects modeling of repeated measures, logarithmic (ln)-transformed blood Pb was statistically significantly associated with an increase in serum creatinine from the previous to current follow-up period in the 428 participants whose highest blood Pb level was ≤ 25 μg/dL (β= 0.027 mg/dL [95% CI: 0.0, 0.054] per unit increase in logarithmic (ln)-transformed blood Pb); effect estimates in the entire group and subsets with different peak blood Pb levels (≤ 10 or 40 μg/dL) also were positive (and larger for blood Pb levels ≤ 10 μg/dL).

This study made key contributions to the understanding of potential uncertainties. In order to address the question of whether nephrotoxicity observed at current blood Pb levels is due to higher blood Pb levels from past exposure, these authors performed a sensitivity analysis in participants whose peak blood Pb levels, dating back to 1979, were

≤ 10 µg/dL. A statistically significant positive association between blood Pb and concurrent serum creatinine remained in a cross-sectional analysis. These authors evaluated reverse causality, which attributes increased blood Pb levels to lack of kidney excretion rather than as a causative factor for CKD, by showing in adjusted plots that the association between blood Pb and serum creatinine occurred over the entire serum creatinine range (0.7-2.1 mg/dL), including the normal range where reverse causality is less likely.

In the subsequent NAS publication (Tsaih et al., 2004), cortical and trabecular bone Pb measurements were obtained in addition to whole blood Pb in evaluations performed in the NAS between 1991 and 1995. Associations between baseline blood, tibia, and patella Pb and change in serum creatinine over an average of 6 years in 448 men were reported. Mean (SD) age, blood Pb level, and serum creatinine at baseline were 66.0 (6.6) years, 6.5 (4.2) µg/dL, and 2.25 (0.2) mg/dL, respectfully. Mean blood Pb levels and serum creatinine decreased significantly over the follow-up period in the whole study group. At baseline, eligible participants were similar to nonparticipants with regard to age, BMI, alcohol consumption, smoking status, diabetic status, hypertensive status, baseline serum creatinine, and blood and bone Pb levels, indicating lack of selective follow-up by blood/bone Pb level or kidney function. Notably, both patella and tibia Pb, which are better markers of long-term exposure than blood Pb in populations that are not currently exposed, but not baseline blood (cross-sectional analyses) Pb level, were positively but nonsignificantly associated with serum creatinine. All three biomarkers of Pb exposure from the entire study group (N =448) were positively associated with longitudinal increases in serum creatinine: blood Pb µg/dL (β = 0.009 mg/dL [95% CI: -0.0008, 0.0188]); tibia Pb µg/g (β = 0.007 mg/dL [95% CI: -0.0028, 0.0168]); and patella Pb µg/g (β = 0.001 mg/dL [95% CI: -0.00684, 0.00884]).

At baseline 6 and 26% of subjects had diabetes and hypertension, respectively. Diabetes was observed to be an effect modifier of the relationship between blood and tibia Pb with 10-year change in serum creatinine. Per unit increase in logarithmic (ln)-transformed blood Pb, the increase in serum creatinine between follow-up periods was substantially stronger in diabetics (β = 0.076 mg/dL [95% CI: 0.031, 0.121]) compared to non-diabetics (β = 0.006 mg/dL [95% CI: -0.004, 0.016]). A similar relationship was observed for tibia Pb. An interaction was also observed between tibia Pb and hypertension, although it is possible that many of the 26 diabetics were also included in the hypertensive group and were influential there as well.

A sensitivity analysis was conducted to characterize the potential for reverse causality by examining participants whose serum creatinine was < 1.5 mg/dL. The authors reported that longitudinal associations did not materially change, and stated that “These

observations in combination with the prospective study design support the conclusion that the direction of the association is lead dose resulting in renal dysfunction". However, the potential for reverse causality still cannot be ruled out.

In modeling the association between blood Pb level and change in serum creatinine, Tsaih et al. (2004) adjusted for baseline serum creatinine. Glymour et al. (2005) discusses how such adjustment may introduce bias. If there is no interaction between Pb exposure and unmeasured causes of kidney disease, the model is linear, and the slope does not change direction prior to and during the study period, the bias should be to underestimate the effect. However, Glymour (2012), noted that the direction of the bias is difficult to predict when the model is nonlinear or the data are restricted to a specific stratum of outcome.

In addition to these analyses of Pb-related increases in serum creatinine among the NAS population, several cross-sectional studies evaluated GFR and creatinine clearance in other populations. The Cadmibel Study was the first large environmental study of this type that adjusted for multiple kidney risk factors, including urinary Cd (Staessen et al., 1992). It included 965 men and 1,016 women recruited from Cd exposed and control areas in Belgium. Mean concurrent blood Pb was 11.4 µg/dL (range 2.3-72.5), and 7.5 µg/dL (range 1.7-60.3) in men and women, respectively. After adjustment for covariates (Table 4-25), log transformed blood Pb was negatively associated with measured creatinine clearance. A 10-fold increase in blood Pb was associated with a decrease in creatinine clearance of 10 and 13 mL/min in men and women, respectively. Blood Pb was also negatively associated with estimated creatinine clearance calculated from the serum creatinine level.

In addition, a cross-sectional study of Swedish women reported that higher concurrent blood Pb (median: 2.2 µg/dL) levels were associated with lower eGFR based on serum cystatin C alone (without age, sex, and race) after adjustment for socio-demographic and CKD risk factors (Akesson et al., 2005). Although GFR was estimated in this study, associations observed in this study were comparable to those using creatinine clearance as the kidney outcome for Pb. In addition, Payton et al. (1994) found a reduction in log-transformed creatinine clearance (mL/min) with increasing blood Pb level among the men enrolled in the NAS.

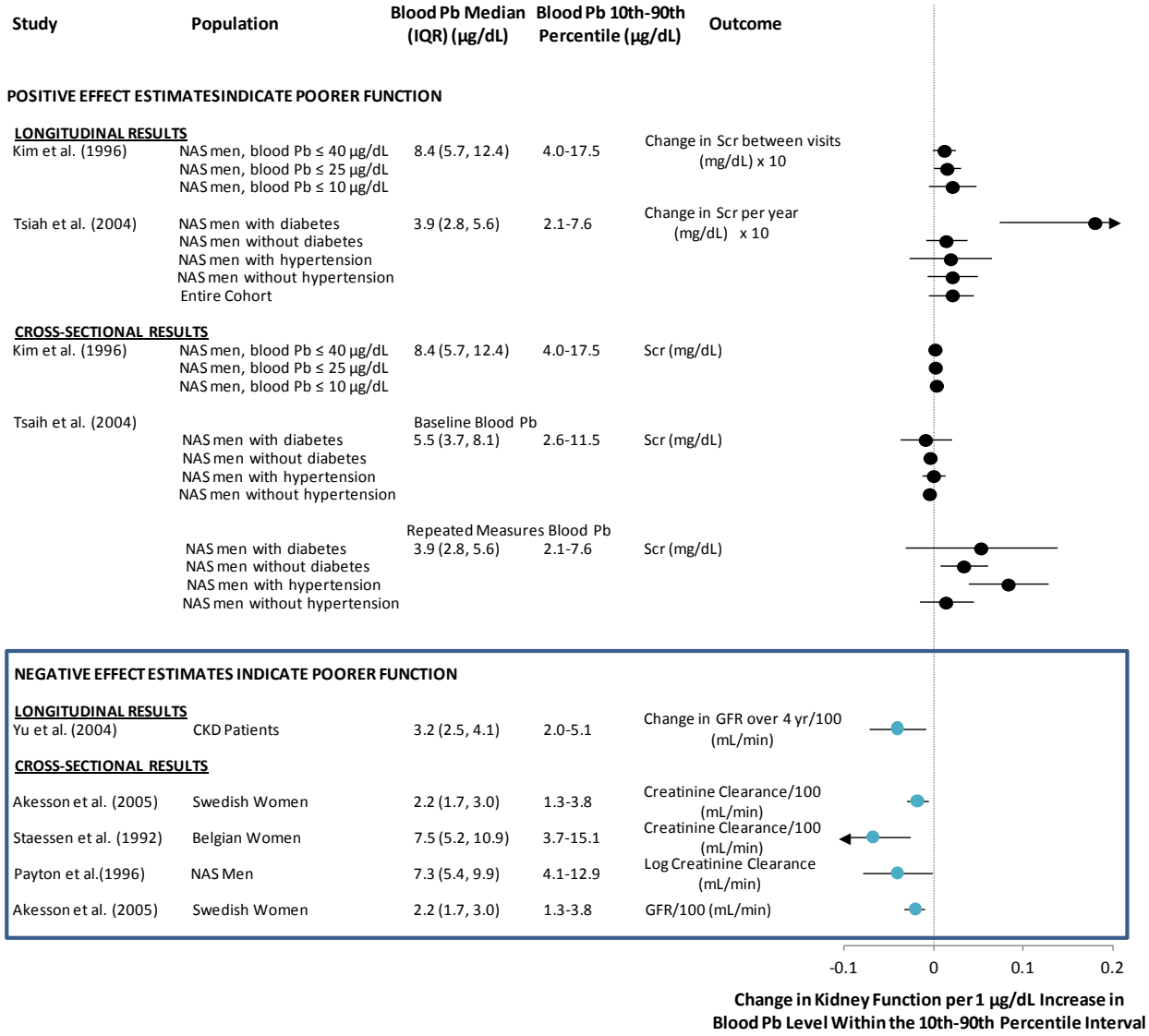
No significant associations between blood Pb level and kidney function were observed in a population of adults (N = 168) and children (described in Section 4.5.2.2) living around smelters in France; however, beta coefficients were not reported (De Burbure et al., 2003). Blood Pb levels were significantly increased in women (mean [SD] control: 4.2 [0.2] µg/dL; polluted: 5.3 [0.18] µg/dL) but not men (mean [SD] control: 7.1 [0.18] µg/dL; polluted: 6.8 [0.17] µg/dL) living in the polluted area.

A longitudinal study of GFR in CKD patients that reported a reduction in GFR with increasing Pb level (Yu et al., 2004) is discussed below in the section Patient Population Studies. Studies evaluating the associations between Pb levels and kidney function in children in the general population are described in detail in Section 4.5.2.2.

The effect of Pb on the kidney has been examined in multiple NHANES datasets obtained over the last few decades (Figure 4-31 and Table 4-25). NHANES data analyses benefit from a number of strengths including large sample size, ability to adjust for numerous potential confounding factors, and the fact that the study population is representative of the U.S. non-institutionalized, civilian population. The results, covering different time frames, have been consistent in providing support for Pb as a CKD risk factor, including NHANES III, conducted from 1988-1994, in which adults with hypertension and diabetes were observed to be potentially at-risk populations (Muntner et al., 2003) and NHANES 1999-2002 (Muntner et al., 2005). However, because the various NHANES analyses were cross-sectional in design, examining associations between concurrent measures of kidney function and blood Pb levels, a common limitation is the uncertainty regarding the temporal sequence between Pb exposure and renal function and the magnitude, timing, frequency, and duration of Pb exposure that contributed to the observed associations.

A recent publication examined NHANES data collected from 1999 through 2006 (Navas-Acien et al., 2009). The geometric mean concurrent blood Pb level was 1.58 μg/dL in 14,778 adults aged ≥ 20 years. After adjustment for survey year, sociodemographic factors, CKD risk factors, and blood Cd, the odds ratios for albuminuria (≥ 30 mg/g creatinine), reduced eGFR (< 60 mL/min/1.73 m^2), and both albuminuria and reduced eGFR were 1.19 (95% CI: 0.96, 1.47), 1.56 (95% CI: 1.17, 2.08), and 2.39 (95% CI: 1.31, 4.37), respectively, comparing the highest (>2.4 μg/dL) to the lowest (≤ 1.1 μg/dL) blood Pb quartiles. Thus, in the subset of the population with the most severe kidney disease (both reduced eGFR and albuminuria), the magnitude of association with concurrent blood Pb was greater. When blood Cd was included as a covariate, blood Pb remained significantly associated with renal function. In fact, the most important contribution of this recent NHANES analysis was the evaluation of joint Pb and Cd exposure (discussed in Section 4.5.4.1).

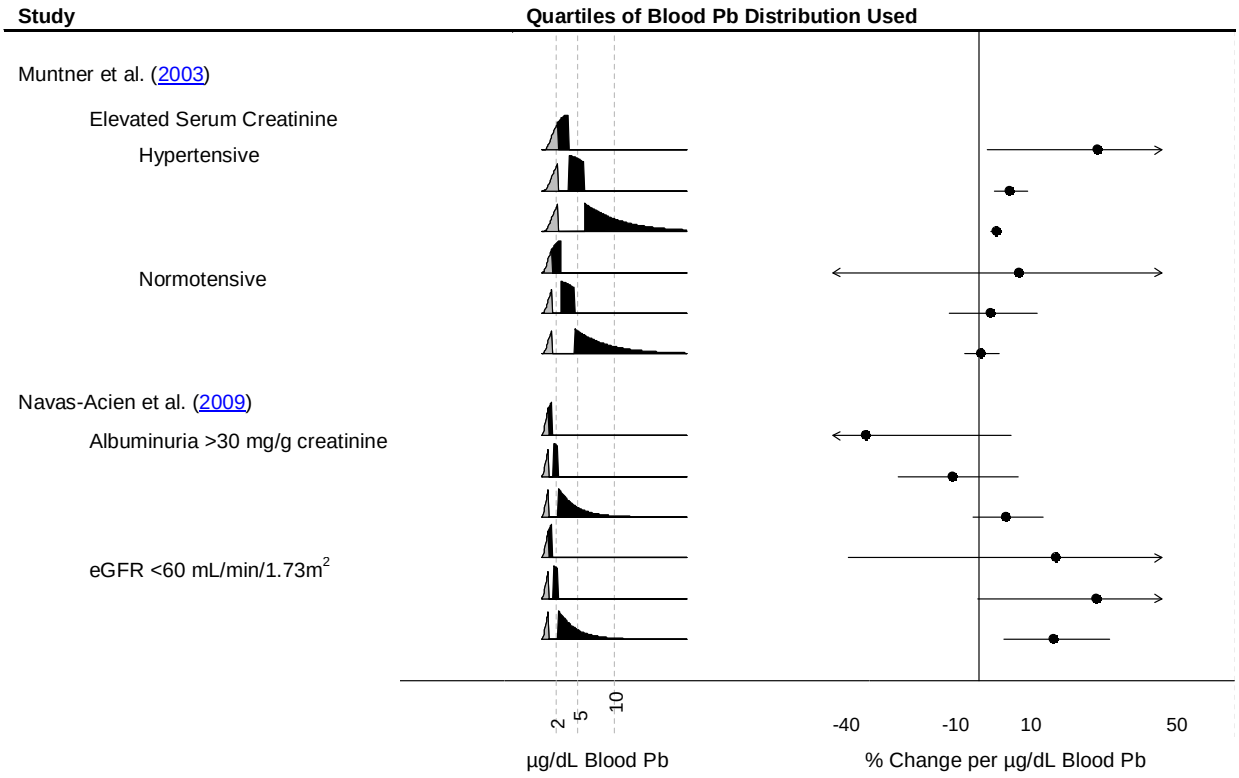
An important contribution of all NHANES publications is that they provide evidence that blood Pb remains associated with reduced kidney function (< 60 mL/min/1.73 m^2 as estimated with the MDRD equation cross-sectionally) despite steadily declining blood Pb levels in the U.S. population during the time periods covered. Other studies of adults participating in NHANES have also reported worse kidney function related to blood Pb levels (Lai et al., 2008a; Hernandez-Serrato et al., 2006; Goswami et al., 2005).



[a]Blood Pb data are presented as median and (IQR) in µg/dL for blood Pb. For uniform presentation, median and IQR were estimated from the given distributional statistics by assuming normal distributions.

Note: Results are presented first for kidney function tests where an increase is considered impaired function (black circles) then for tests where a decrease is considered impaired function (blue circles, outlined in box). Within a category, results are presented first for longitudinal analyses followed by cross-sectional analyses. To compare results for linear and nonlinear modeling, effect estimates were standardized to a 1 µg/dL increase in blood Pb level within the 10th-90th percentile interval. Magnitudes of the effect should not be compared among different kidney metrics.

Figure 4-30 Concentration-response relationships for associations between blood Pb level and kidney function outcomes in adults.



Note: These results depicted are from studies that reported ORs of kidney function measures by grouping the population into quartiles of blood Pb and then comparing each group to the quartile with the lowest blood Pb (reference group). The blood Pb distribution of the examined group is shaded black and the reference group is shaded gray. To express these odds ratios in terms of blood Pb concentration, a log normal distribution was fit to the statistics presented and then the medians of each group were determined. The adjusted OR was the exponentiated quantity (log(OR) divided by the difference in the medians of the groups compared). The resulting odds ratio is presented in terms of percent change=100*(OR-1).

Figure 4-31 Percent change in kidney outcomes across quartiles of blood Pb level in NHANES.

Summary of General Population Studies

Collectively, the general population studies summarized in Figure 4-30, Figure 4-31 and Table 4-25 provide critical evidence on the effects of Pb exposure on the kidney. Two longitudinal studies provide high quality evidence for an association between reduced kidney function and blood Pb level with adjustment for numerous potential confounding factors. As presented in Table 4-25 the slopes and 95% CI limits per 1 µg/dL increase in blood Pb level are similar in magnitude for the entire population assessed by Tsaih et al. (2004) and for the group of individuals with a peak blood Pb level ≤ 10 µg/dL assessed by Kim et al. (1996). These studies show an overall pattern of elevated risks across all

subgroups analyzed within the population. However, because blood Pb level in nonoccupationally-exposed adults reflects both recent and past Pb exposures, the magnitude, timing, frequency, and duration of Pb exposure contributing to the observed associations is uncertain.

Although uncertainties remain, the longitudinal analyses of the NAS populations included sensitivity analyses designed to address the potential for reverse causality to explain findings. Furthermore, these general population studies included adjustment for multiple key potential confounding factors including age, race, sex, education, household income, smoking, alcohol use, and various health indicators such as diabetes, systolic BP, BMI, and history of cardiovascular disease. Exposure to copollutants such as Cd was also adjusted (Staessen et al., 1992).

Although the cross-sectional studies do not consistently report positive and significant findings, they are compatible with evidence from higher quality studies of an effect of long-term Pb exposure on kidney function. The strengths and limitations of studies conducted in other populations are discussed in the sections that follow.

Table 4-25 Additional characteristics and quantitative data for associations of blood and bone Pb with kidney outcomes for results presented in Figure 4-30 and Figure 4-31.

Reference	Population Study Location Time Period	Pb Biomarker Data	Outcome	Statistical Analysis	Effect Estimate (95% CI)
Results for Figure 4-30: Positive Effect Estimates Indicate Poorer Function					**Per 1 µg/dL increase in blood Pb within the 10th-90th percentile interval**
Longitudinal Results					
Kim et al. (1996)	Adult males N=459 Boston, MA; Multiple examinations 1979-1994	Median baseline blood Pb: 8.6 µg/dL 10th-90th percentile: 4.0-17.5	Change in serum creatinine between visits x 10 (mg/dL)	Random-effects modeling adjusted for baseline age, time since initial visit, BMI, smoking status, alcohol ingestion, education level, hypertension, baseline serum creatinine, and time between visits	Peak blood Pb ≤ 40 µg/dL: 0.012 (-0.0001, 0.025) Peak blood Pb ≤ 25 µg/dL: 0.015 (0.0002, 0.03) Peak blood Pb ≤ 10 µg/dL: 0.021 (-0.005, 0.048)

Table 4-25 (Continued): Additional characteristics and quantitative data for associations of blood and bone Pb with kidney outcomes for results presented in Figure 4-30 and Figure 4-31.

Reference	Population Study Location Time Period	Pb Biomarker Data	Outcome	Statistical Analysis	Effect Estimate (95% CI)
Tsaih et al. (2004)	Adult males N=448 Boston, MA; 8/1991-1995 with mean 6 year follow-up	Mean (SD) baseline Blood Pb: 6.5 (4.2) µg/dL 10th-90th percentile: 2.1-7.6 Tibia Pb: 21.5 (13.5) µg/g Patella Pb: 32.4 (20.5) µg/g	Change in serum creatinine per year x 10 (mg/dL)	Log linear regression adjusted for age, age squared, BMI, hypertension, diabetes, smoking status, alcohol consumption, analgesic use, baseline serum creatinine, serum creatinine squared	Entire Cohort (N=448) 0.0210 (-0.0019, 0.048) With diabetes: 0.18 (0.07, 0.29) Without diabetes: 0.014 (-0.009, 0.037) With hypertension: 0.019 (-0.027, 0.065) Without hypertension: 0.021 (-0.007, 0.049) Per unit increase in logarithmic (ln)-transformed tibia Pb Entire Cohort (N=448) 0.007 (-0.0028, 0.0168) With diabetes: 0.082 (0.03, 0.14) Without diabetes: 0.005 (-0.01, 0.02) With hypertension: 0.023 (0.003, 0.04) Without hypertension: 0.0004 (-0.01, 0.01)
Cross-Sectional Results					
Kim et al. (1996)	Adult males N=459 Boston, MA; Multiple examinations 1979-1994	Median baseline blood Pb: 8.6 µg/dL 10th-90th percentile: 4.0-17.5	Serum creatinine (mg/dL)	Random-effects modeling adjusted for baseline age, time since initial visit, BMI, smoking status, alcohol ingestion, education level, and hypertension.	Peak blood Pb ≤ 40 µg/dL: 0.0017 (0.0005, 0.003) Peak blood Pb ≤ 25 µg/dL: 0.0021 (0.0007, 0.0035) Peak blood Pb ≤ 10 µg/dL: 0.0033 (0.0012, 0.0053)

Table 4-25 (Continued): Additional characteristics and quantitative data for associations of blood and bone Pb with kidney outcomes for results presented in Figure 4-30 and Figure 4-31.

Reference	Population Study Location Time Period	Pb Biomarker Data	Outcome	Statistical Analysis	Effect Estimate (95% CI)
Tsaih et al. (2004)	Adult males N = 448 Boston, MA; 8/1991-1995 with mean 6 yr follow-up	Mean (SD) baseline Blood Pb: 6.5 (4.2) µg/dL 10th-90th percentile: 2.6-11.5 Repeated measures 10th-90th percentile: 2.1-7.6 Tibia Pb: 21.5 (13.5) µg/g Patella Pb: 32.4 (20.5) µg/g	Serum creatinine (mg/dL)	Log linear regression adjusted for age, age squared, BMI, hypertension, diabetes, smoking status, alcohol consumption, analgesic use	Baseline blood Pb With diabetes: -0.009 (-0.038, 0.020) Without diabetes: -0.004 (-0.010, 0.003) With hypertension: 0 (-0.013, 0.013) Without hypertension: -0.005 (-0.011, 0.002) Follow-up blood Pb With diabetes: 0.053 (-0.032, 0.138) Without diabetes: 0.034 (0.007, 0.061) With hypertension: 0.083 (0.038, 0.128) Without hypertension: 0.014 (-0.016, 0.044)
Results for Figure 4-30: Negative Effect Estimates Indicate Poorer Function					
Longitudinal Results:					**Per 1 µg/dL increase in blood Pb within the 10th-90th percentile interval**
Yu et al. (2004)	Adult CKD patients N = 121 Taipei, Taiwan; 48 month longitudinal study period	Mean (SD) baseline blood Pb: 4.2 (2.2) µg/dL 10th-90th percentile: 2.0-5.1	Change in MDRD eGFR over 4 yr/100 (mL/min/1.73 m^2 body surface area)	Cox proportional hazard model examined whether a predictor was associated with renal function including age, sex, BMI, hyperlipidemia, hypertension, smoking, use of ACE inhibitor, baseline serum creatinine, daily protein excretion, daily protein intake, underlying kidney disease	-0.040 (-0.072, -0.008)[a]

Table 4-25 (Continued): Additional characteristics and quantitative data for associations of blood and bone Pb with kidney outcomes for results presented in Figure 4-30 and Figure 4-31.

Reference	Population Study Location Time Period	Pb Biomarker Data	Outcome	Statistical Analysis	Effect Estimate (95% CI)
Cross-Sectional Results:					
Akesson et al. (2005)	WHILA, adult women N = 820 Sweden; 6/1999-1/2000	Median (5-95% CI) concurrent blood Pb: 2.2 (1.1, 4.6) µg/dL 10th-90th percentile: 1.3-3.8	Creatinine clearance/100 (mL/min)	Linear regression adjusted for age, BMI, diabetes, hypertension, regular use of nephrotoxic drug, smoking status	-0.018 (-0.03, -0.006)
			Cystatin C-based eGFR (Larsson et al., 2004)/100 (mL/min)		-0.02 (-0.03, 0.007)
Staessen et al. (1992)	Adults N =1,981 Belgium; 1985-1989	Concurrent blood Pb mean (SD) Males: 11.4 µg/dL Females: 7.5 µg/dL 10th-90th percentile: 3.7-15.1	Creatinine clearance/100 (mL/min)	Log linear regression adjusted for age, age squared, sex, BMI, BP, ferritin level, smoking status, alcohol ingestion, rural/urban residence, analgesic and diuretic use, blood and urinary Cd, diabetes, occupational exposure to heavy metals, and gamma glutamyl transpeptidase	Females: -0.067 (-0.108, -0.027)[a] Males: -0.051 (-0.097, -0.047)[a]
Payton et al. (1994)	Adult males N =744 Boston, MA; 1988-1991	Mean (SD) concurrent blood Pb: 8.1 (3.9) µg/dL 10th-90th percentile: 4.1-12.9	Log-transformed creatinine clearance (mL/min)	Log linear regression adjusted for age, BMI, analgesic and diuretic use, alcohol consumption, smoking status, SBP, DBP	-0.040 (-0.079, -0.0015)

Table 4-25 (Continued): Additional characteristics and quantitative data for associations of blood and bone Pb with kidney outcomes for results presented in Figure 4-30 and Figure 4-31.

Reference	Population Study Location Time Period	Pb Biomarker Data	Outcome	Statistical Analysis	Effect Estimate (95% CI)
Results for Figure 4-31: Analysis of Blood Pb Quartiles					**% change in kidney outcome**
Muntner et al. (2003)	NHANES III, adults N = 4,813 U.S.; 1988-1994	Mean (SD) concurrent blood Pb: With Hypertension: 4.2 (0.14) µg/dL Q1: 0.7 to 2.4 Q2: 2.5 to 3.8 Q3: 3.9 to 5.9 Q4: 6.0 to 56.0 Without Hypertension: 3.3 (0.10) µg/dL Q1: 0.7 to 1.6 Q2: 1.7 to 2.8 Q3: 2.9 to 4.6 Q4: 4.7 to 52.9	Elevated Serum Creatinine (99th percentile of each race-sex specific distribution for healthy young adults) CKD	Logistic regression adjusted for age, race, sex, diabetes, SBP, smoking, history of CVD, BMI, alcohol consumption, household income, education level, marital status, health insurance	Q1: Referent With hypertension Q2: 47% (3, 110) Q3: 80% (34, 142) Q4: 141% (46, 297) Without hypertension Q2: 11% (-44, 121) Q3: 19% (-38, 125) Q4: 9% (-47, 122) With hypertension Q2: 44% (0, 109) Q3: 85% (32, 159) Q4: 160% (52, 345) Without hypertension Q2: -10% (-63, 116) Q3: 0% (-55, 122) Q4: 9% (-59, 189)
Navas-Acien et al. (2009)	NHANES III, adults N = 14,778 U.S.; 1999-2006	Geometric mean concurrent blood Pb: 1.58 µg/dL Q1: ≤ 1.1 Q2: 1.2 to 1.6 Q3: 1.7 to 2.4 Q4: >2.4	eGFR <60 mL/minute/1.73 m^2 Albuminuria and eGFR <60 mL/minute/1.73 m^2	Logistic regression adjusted for survey year, age, sex, race/ethnicity, BMI, education, smoking, cotinine, alcohol intake, hypertension, diabetes, menopausal status	Q1: Referent Q2: 10% (-20, 51) Q3: 36% (-1, 85) Q4: 56% (17, 108) Q2: 53% (-15, 177) Q3: 57% (-17, 198) Q4: 139% (31, 337)

[a]95% CI: estimated from given p-value.

Patient Population Studies

CKD as defined by the National Kidney Foundation (NKF) - Kidney Disease Outcomes Quality Initiative workgroup (NKF, 2002) is the presence of markers of kidney damage or GFR < 60 mL/min/1.73 m^2 for ≥ 3 months. The MDRD equation is the most common one used in the eGFR determination for this definition. Notably, decreased GFR is not required for the first criterion and markers of kidney damage are not required for the second criterion.

Several key studies in CKD patients provide prospective data that indicate that higher baseline blood Pb level is associated with greater CKD progression over time (kidney function decline) in patient populations (Table 4-26). Yu et al. (2004), discussed in the 2006 Pb AQCD, followed 121 patients over a four year period. Eligibility required well-controlled CKD with serum creatinine between 1.5 and 3.9 mg/dL. Importantly, EDTA-chelatable Pb < 600 μg/72 hours, a level below that traditionally thought to indicate risk for Pb-related nephrotoxicity, was required at baseline. Patients with potentially unstable kidney disease were excluded (i.e., due to systemic diseases such as diabetes). Mean blood Pb and EDTA-chelatable Pb levels were 4.2 μg/dL and 99.1 μg/72 hours, respectively. Cox proportional hazard modeling indicated lack of significant association between serum creatinine changes and various potential confounding factors (Table 4-25), examined one at a time. Only chelatable Pb (body Pb burden indicator) was significantly associated with overall risk for the primary endpoint (doubling of serum creatinine over the 4-year study period or need for hemodialysis). When the group was dichotomized by EDTA chelatable Pb level, Kaplan-Meier analysis demonstrated that significantly more patients (15/63) in the high-normal group (EDTA chelatable Pb level ≥ 80 but < 600 μg/72 hours) reached the primary end point than did those in the lower EDTA chelatable Pb levels (<80 μg Pb/72 hours) group (2/58). Associations between baseline chelatable or blood Pb level and change in serial measurements of eGFR (estimated by the MDRD equation (Levey et al., 1999)) were modeled separately using generalized estimating equations. Based on these models, a 10 μg higher chelatable Pb level or 1 μg/dL higher blood Pb level reduced the GFR by 1.3 and 4.0 mL/min/1.73 m^2, respectively, during the 4-year study period. The use of estimated GFR provides a better estimate of progressive changes of renal function than creatinine clearance used in the other related studies.

Recent studies expanded the CKD patient populations in which this effect was observed to include those with diabetic nephropathy (Lin et al., 2006b) and with the lowest blood Pb levels studied to date (Lin et al., 2006a). A potential limitation in these studies is lack of researcher blinding. The treatment protocol, which included additional calcium

disodium EDTA infusions as needed based on Pb levels during follow-up, may differ from the placebo protocol; therefore, patients may have been able to discern their treatment group. In addition, it is possible that the improved renal function following the lowering of blood Pb level by chelation is a result of the secondary effect of reducing ROS, blood Pb level or both. Results of these observational studies have been summarized in Table 4-26 (Weaver and Jaar, 2010).

Table 4-26 Prospective patient population studies: kidney function decline.

Study	N	Baseline mean (SD) blood Pb (μg/dL)	Baseline mean (SD) chelatable Pb (μg/72 hours)	Baseline mean(SD) eGFR (mL/min /1.73 m^2)	Years of follow-up	Decline in eGFR per 1 SD higher Pb dose at baseline per year	Comments
Lin et al. (2003)	202	5.3 (2.9)	104.5 (106.3)	41.6 (14.4)	2	0.16	Largest study to date
Yu et al. (2004)	121	4.2 (2.2)	99.1 (83.4)	36.0 (9.8)	4	2.7 (chelatable) 2.2 (blood Pb)	Longest follow-up; 1 μg/dL higher blood Pb, at baseline, associated with 4.0 mL/min/1.73 m^2 reduction in eGFR over 4 years
Lin et al. (2006b)	87	6.5 (3.4)	108.5 (53.8)	35.1 (9.0)	1	3.87	Type II diabetics with nephropathy
Lin et al. (2006a)	108	2.9 (1.4)[a]	40.2 (21.2) (all <80)	47.6 (9.8)	2	1.1	Lowest Pb exposed CKD patients

[a]Notably, mean blood Pb level in this study was below that observed in a recent large general population study of 50- to 70-year olds in Baltimore, MD (Martin et al., 2006).

Source: Reprinted with permission of UpToDate.com, Weaver and Jaar (2010)

A recent population-based case-control study examined occupational Pb exposure as a risk factor for severe CKD (Evans et al., 2010). The study included 926 cases with first time elevations of serum creatinine >3.4 mg/dL for men and >2.8 mg/dL for women and 998 population-based controls. Occupational Pb exposure was assessed using an expert rating method based on job histories; no biomarkers of Pb exposure were measured. In multivariable logistic regression modeling, the OR for CKD (adjusted for age, sex, smoking, alcohol consumption, diabetes, education, and BMI) was negative but not significant (0.97 [95% CI: 0.68, 1.38] in Pb-exposed compared to non-exposed participants). In addition, the CKD patients were followed prospectively for a mean of 2.5 years for the 70 Pb-exposed patients and 2.4 years for the 731 patients without past

occupational Pb exposure. Mean eGFRs (using the MDRD equation) were 16.0 and 16.6 mL/min/1.73 m^2 in exposed and non-exposed patients, respectively, indicating severe disease in both groups. The authors caution that although the high serum creatinine limit (exclusion criteria: 3.4 mg/dL for men and 2.8 for women), enough to ensure both high sensitivity and specificity, is a strength in the case-control analyses, it is a limitation in the prospective follow up analysis; they note the further limitations that serum creatinine was not measured in controls and the absence of blood Pb measurements. The expert ratings used in this study may have lower validity and reliability as compared to other exposure assessment methods (Teschke et al., 2002) including blood and bone measurements used in the majority of well-conducted studies. The lack of blood or bone Pb exposure estimates in this study compared to other studies considered suggests placing reduced weight on this study.

Strengths included virtually complete case ascertainment and minimal loss to follow-up. Exposure assessment was presented by the authors as both a strength and a limitation. Expert rating methods are commonly used when biological monitoring is not an option and in case-control studies where many occupational exposures are considered. In Pb-kidney research, this approach is uncommon except in the case-control setting. However, given the challenges of interpreting blood Pb in dialysis patients (discussed below), this approach may have advantages in this study of such severe CKD. Other case-control studies examining occupational risk factors for CKD found Pb exposure to be a risk factor (Nuyts et al., 1995; Steenland et al., 1990). Nuyts et al. (1995) found adults with history of occupational Pb exposure to have elevated odds of CKD (OR for ever- versus never-exposed: 2.11 [95% CI: 1.23, 4.36]). The association was weaker in Steenland et al. (1990) (OR for ever- versus never-exposed: 1.73 [95% CI: 0.82, 3.65]). Regular moonshine consumption, also a potential source of Pb exposure, was a stronger risk factor for CKD (OR: 2.42 [95% CI: 1.10, 5.36]).

The prospective observational aspect of Evans et al. (2010) is similar in design to the work of Lin and colleagues but differs in several important respects. In Evans et al. (2010), only occupational Pb exposure was considered whereas the work in Taiwan excluded occupational exposure and used blood and chelatable Pb measures. In the past in developed countries, environmental exposures were substantial. For example, mean tibia Pb levels were 21.5 and 16.7 μg/g bone mineral, in environmentally-exposed 50- to 70-year-old African-Americans and whites, respectively, in Baltimore (Martin et al., 2006). In Korean Pb workers, mean baseline tibia Pb level was only twofold higher (35.0 μg/g) (Weaver et al., 2003a) which illustrates the substantial body burden in middle- and older-aged Americans from lifetime Pb exposure. Declines in blood Pb levels in Sweden have been reported and attributed to the leaded gasoline phase-out (Strömberg et al., 1995; Elinder et al., 1986), although blood Pb levels were lower than those noted during

the U.S. phase-out. Finally, the severe degree of CKD among subjects in Evans et al. (2010) creates a survivor bias at enrollment and limits the eGFR decline possible during follow-up, thus limiting the ability to identify factors that influence that decline.

ESRD Patient Studies

End stage renal disease (ESRD) is a well-established public health concern, and is characterized by the use of dialysis to perform the normal functions of the kidney. Incidence and prevalence in the U.S. continue to increase resulting in rates that are the third highest among nations reporting such data (U.S. Renal Data System, 2009). Studies in patients with CKD requiring chronic hemodialysis have also been published in the past five years. A study of 271 adult patients on regular thrice weekly dialysis reported much higher blood Pb levels than had been appreciated by the treating clinicians (Davenport et al., 2009). Blood Pb levels ranged from 3 to 36.9 μg/dL; 25.5% had levels >20 μg/dL, 59% had values of 10-20 μg/dL, and 15.5% were <10 μg/dL. Few details on the statistical analysis were provided which complicates interpretation of the findings. However, blood Pb was positively correlated with hemodialysis vintage (months on dialysis; Spearman $r = 0.38$, $p < 0.001$); negatively correlated with urine output ($r = -0.44$, $p < 0.001$) and higher in patients using single carbon filter and reverse osmosis water purification devices. Another recent publication reported higher Pb in dialysate than in the tap water used in its preparation (Chen et al., 2009a). A systematic review of a wide range of trace elements in hemodialysis patients reported higher Pb levels in patients compared to controls although the difference was not large (Tonelli et al., 2009). These data suggest that blood Pb monitoring in dialysis patients may be useful.

Interpretation of blood and bone Pb in patients on dialysis is challenging for several reasons. First, renal osteodystrophy, the bone disease related to kidney disease, may result in increased release of Pb from bone stores. Thus, interpretation of blood and even bone Pb levels may require adjustment with one or more of a range of osteoporosis variables. Secondly, as observed above (Davenport et al., 2009), residual kidney function may have a substantial impact on blood Pb levels in populations with such minimal excretion. Third, as illustrated in the studies cited above (Chen et al., 2009a; Davenport et al., 2009), water and concentrates used in dialysis may be variable sources of Pb. A recent study reported decreased blood Pb in post-dialysis compared to pre-dialysis samples (Kazi et al., 2008). Thus, substantial fluctuations in blood Pb are possible while on dialysis. Finally, anemia is common in CKD and Pb is stored in red blood cells. Thus, measurement of blood Pb in anemia may require adjustment for hemoglobin; no standardized approach to this currently exists.

Given these caveats, a small cross-sectional pilot study observed higher median blood Pb levels in 55 African-American dialysis patients compared to 53 age- and sex-matched controls (6 and 3 μg/dL respectively; p <0.001) (Muntner et al., 2007). However, median tibia Pb was higher in ESRD patients although the difference did not reach statistical significance (17 and 13 μg/g bone mineral, respectively [p = 0.13]). Further, the authors note the limitation related to the sample size based on too few cases needed to achieve statistical significance from power calculations.

In order to determine the potential impact of renal osteodystrophy, median blood and tibia Pb levels in the dialysis patients were compared by levels of serum parathyroid hormone, calcium, phosphorus, and albumin and were not found to be significantly different (Ghosh-Narang et al., 2007). A study of 211 diabetic patients on hemodialysis (Lin et al., 2008) found parathyroid hormone and serum creatinine to be associated with blood Pb level in crude but not adjusted associations. In contrast, a study of 315 patients on chronic peritoneal dialysis observed parathyroid hormone to be positively correlated and residual renal function to be negatively correlated with logarithmic-transformed blood Pb levels after adjustment (Lin et al., 2010). In the prospective portion of this study, blood Pb levels at baseline were categorized by tertile (range of 0.1 to 29.9 μg/dL with cut points of 5.62 and 8.66 μg/dL). Cox multivariate analysis, after adjustment for parathyroid hormone level, residual renal function, and 20 other variables, showed increased all-cause mortality in the middle (5.62-8.66 μg/dL) and highest (>8.66 μg/dL) compared to the lowest (<5.62 μg/dL) tertiles after 18 months of follow-up (hazard ratio= 2.1 [95% CI: 2.0, 2.2] and 3.3 [95% CI: 1.3, 13.5], respectively). A recent publication of an 18-month follow-up of 927 patients on maintenance hemodialysis also reported increased hazard ratios for all-cause (4.7 [95% CI: 1.9, 11.5]), cardiovascular-cause (9.7 [95% CI: 2.1, 23.3]), and infection-cause (5.4 [95% CI: 1.4, 20.8]) 18-month mortality in the highest (>12.64 μg/dL) compared to the lowest tertile (<8.51 μg/dL) of baseline blood Pb level, after adjustment for sex, urban residence, hemodialysis vintage, hemoglobin, serum albumin, and ferritin (Lin et al., 2011). Given other recent publications in hemodialysis patients by this group, it would be valuable to examine these risks after adjustment for hemoglobin A1C (Lin-Tan et al., 2007a), and blood Cd (Yen et al., 2011; Hsu et al., 2009a).

Clinical Trials in Chronic Kidney Disease Patients

Randomized chelation trials in CKD patients, uncommon in nephrotoxicant research, provide unique information on the impact of Pb on the kidney. These studies have been performed by Lin and colleagues at the Chang Gung Memorial Hospital in Taipei, Taiwan and involve similar study designs. Initially, patients were observed to compare CKD progression prior to chelation. Then, CKD patients whose diagnostic EDTA

chelatable Pb levels were within certain ranges (generally 60-600 μg/72 hours and thus below the level commonly considered for chelation) were randomized. The treated group received weekly chelation with 1 g EDTA intravenously for up to 3 months. The control group received placebo infusions. In the follow-up period, chelation was repeated for defined indications such as increased serum creatinine or chelatable Pb levels above specified cut-offs. Placebo infusions were repeated in the controls as well. During the intervention periods single-blind, randomized, placebo-controlled interventions were conducted. The results of the most recent of these trials are summarized in Table 4-27 below.

Table 4-27 Clinical randomized chelation trials in chronic kidney disease patients.

Reference	Group	N	Baseline mean(SD) blood Pb (μg/dL)	Baseline mean(SD) chelatable Pb (μg/72 hr)	Baseline mean(SD) eGFR (mL/min /1.73 m^2)	Months of treatment / follow-up	Change in eGFR per yr (mL/min /1.73 m^2)	Comments
Lin et al. (2003)	Chelated	32	6.1 (2.5)	150.9 (62.4)	32.0 (12.1)	27	+ 1.07	
	Control	32	5.9 (3.0)	144.5 (87.9)	31.5 (9.0)		- 2.7	
Lin et al. (2006b)	Chelated	15	7.5 (4.6)	148.0 (88.6)	22.4 (4.4)	15	-3.5	Subjects with Type II diabetes and nephropathy
	Control	15	5.9 (2.2)	131.4 (77.4)	26.3 (6.2)		-10.6	
Lin et al. (2006a)	Chelated	16	2.6 (1.0)[a]	43.1 (13.7)	41.2 (11.2)	27	+3.0	Lowest Pb exposed and treated range Body Pb Burden (72 h urinary Pb excretion) ≥ 20 to <80 μg
	Control	16	3.0 (1.1)	47.1 (15.8)	42.6 (9.7)		-2.0	
Lin-Tan et al. (2007b)	Chelated	58	5.0 (2.2)	164.1 (111.1)	36.8 (12.7)	51	-0.3	Subjects without diabetes
	Control	58	5.1 (2.6)	151.5 (92.6)	36.0 (11.2)		-2.9	

[a]Notably, mean blood Pb level in this study was below that observed in a recent large general population study of 50- to 70-year olds in Baltimore, MD (Martin et al., 2006).

The unique body of work in patient populations by Lin and coworkers, both observational and experimental, has numerous strengths including prospective study design, randomization, Pb assessment that includes estimates of the bioavailable dose, longitudinal statistical analysis, and control for multiple kidney risk factors. However, the generalizability of the results to broader populations is unknown. In addition, the association observed between Pb dose and decline in GFR has been variable; the annual decline in eGFR per standard deviation higher Pb dose at baseline was much lower in the 2003 study than in subsequent publications (Table 4-27 above). Small sample sizes and differences in renal diagnoses between groups may be factors in this variability.

The studies presented in Table 4-27 had a number of potential limitations. This included small sample size. Also, the researchers were not blinded and the treatment protocol, which included additional calcium disodium EDTA infusions as needed based on Pb levels during follow-up, may differ from the placebo protocol; therefore, patients may be able to discern their treatment group. Another possible limitation may be the shorter follow-up time in some of the studies. The use of creatinine clearance to assess changes in renal function may limit interpretation of results as discussed in Section 4.5.1.1. Also, the effects observed following chelation therapy may result from removal of other ions such as Zn, Cu, and Fe. In addition, changes in kidney function after treatment with Pb chelating agents may be by mechanisms other than reduction in Pb body burden. Chelating agents have been shown to act as antioxidants. DMSA abolished reactive oxygen species formation (i.e., MDA and nitrotyrosine in interlobular arteries) and was protective against non-Pb-induced nephrosclerosis in rats (Gonick et al., 1996). EDTA administration enhanced endothelial NO production and reduced kidney damage in a rat model of ischemia-induced acute renal failure (Foglieni et al., 2006). Improved renal function following administration of chelating agents have been reported in rodent models of Pb-induced nephrotoxicity (Sanchez-Fructuoso et al., 2002a; Sanchez-Fructuoso et al., 2002b; Khalil-Manesh et al., 1992a). Chelation did not appear to improve Pb-induced structural damage (Khalil-Manesh et al., 1992a); again suggesting that improved hemodynamics may be a result of reduction in reactive oxidant species, which could be due to reduced Pb level and/or directly to the chelating agent (Gonick et al., 1996). Despite these uncertainties and limitations, the most plausible explanation for the combination of the observational and experimental chelation work of Lin and colleagues is that reduced Pb is the underlying reason for improved kidney function. The limitations discussed suggest putting less weight on these clinical trials in CKD patient studies. This study design requires replication in larger populations at multiple clinical centers to confirm that the change in renal function may be due to removal of Pb.

Occupational Studies

The vast majority of studies in the literature on the impact of Pb on the kidney have been conducted in the occupational setting. In general, study size and extent of statistical analysis are much more limited than those in general population studies. Publications in few populations have reported adjusted results in occupationally exposed workers in the five years since the 2006 Pb AQCD.

A study of Korean Pb workers included in the 2006 Pb AQCD reported inconsistent results with higher Pb measures associated with worse renal function in some models and better renal function in other models (Weaver et al., 2003a). Null associations were observed when the entire population was studied. In models of effect modification by age, a pattern emerged in which higher Pb exposure and dose measures were associated with worse renal function in older workers and better renal function in younger workers (Weaver et al., 2003a). This cohort was expanded into a two-year prospective cohort study with data from Weaver et al. (2003a) serving as baseline Pb measures (Weaver et al., 2009). Generalized estimating equations were used to model change in kidney function between each evaluation in relation to tibia Pb and concurrent change in blood Pb in 537 current and former Pb workers while adjusting for baseline Pb dose and other covariates (Weaver et al., 2009). Tibia Pb was evaluated at the beginning of each follow-up period (yearly on average) and Pb biomarker levels were adjusted for baseline levels and other covariates. In males, serum creatinine declined and calculated creatinine clearance increased over the course of the study; these changes were largest in participants whose blood Pb declined concurrently or whose tibia Pb was lower at the beginning of the follow-up interval. In females, decreasing serum creatinine was associated with declining blood Pb (as in males); however, increasing blood Pb was associated with a concurrent increase in serum creatinine. Women (25.9% of the study population) were older and more likely to be former Pb workers than were men which may have been important factors in the effect modification observed by sex.

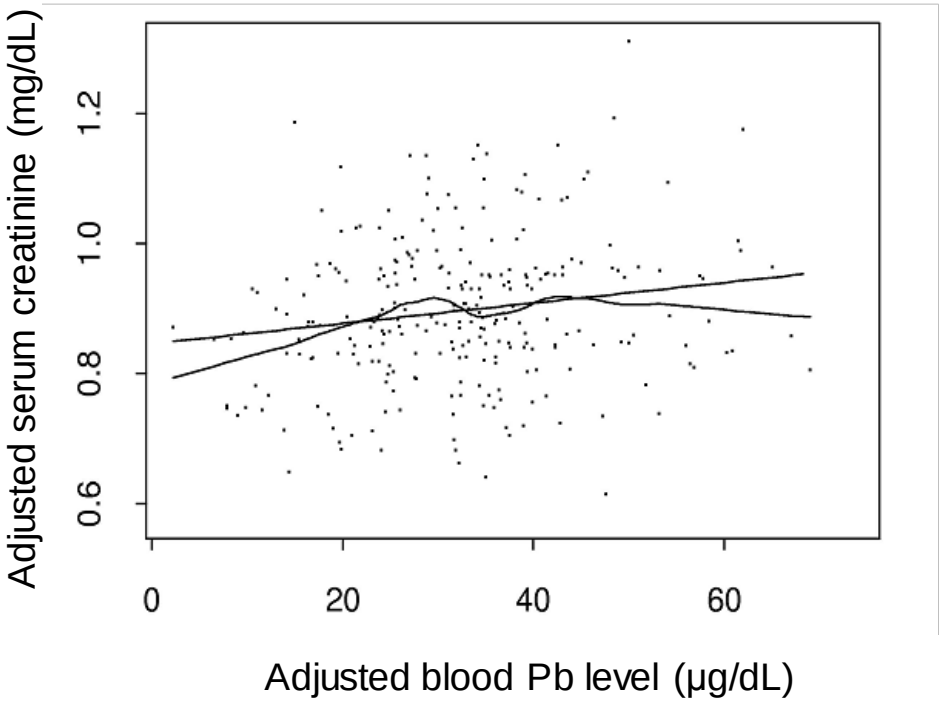
Chia and colleagues observed a significant, positive association between concurrent blood Pb and urine NAG in linear regression models after adjustment for age, sex, race, exposure duration, ALAD G177C polymorphism and the interaction between ALAD genotype and blood Pb (Chia et al., 2006). Similar positive associations were observed between blood Pb and a wider range of EBE markers in models that adjusted for age, sex, race, exposure duration, and the HpyCH4 ALAD polymorphism (Chia et al., 2005). Other studies published in the last 5 years also focused on ALAD polymorphisms but did not find effect modification to be in a consistent direction (Gao et al., 2010a; Wang et al., 2009a; Weaver et al., 2006; Weaver et al., 2005b). In adults with the ALAD2 genotype, Pb has been associated with better and poorer renal function in separate cohorts of Pb workers.

Two studies of occupationally-exposed adults have performed benchmark dose calculations for the effect of Pb on the kidney. Both used only EBE markers and found NAG to be the most sensitive outcome; reported lower confidence limits on the benchmark doses were 10.1 µg/dL (Sun et al., 2008b), and 25.3 µg/dL (Lin and Tai-yi, 2007).

A number of other publications in the five years since the 2006 Pb AQCD, have reported significantly worse kidney outcomes in unadjusted analyses in occupationally-exposed adults compared to unexposed controls (Onuegbu et al., 2011; Patil et al., 2007) and/or significant correlations between higher levels of Pb biomarkers and worse kidney function (Alasia et al., 2010; Khan et al., 2008; Garcon et al., 2007; Lin and Tai-yi, 2007; Alinovi et al., 2005). A study of 155 male workers reported significant, positive correlations between blood and urine Pb and urine NAG and albumin after controlling for age and job duration (Sun et al., 2008b). One small study found no significant differences (Orisakwe et al., 2007). In a study of 108 Pb workers with mean blood Pb level of 36.2 µg/dL, no significant correlations were observed between blood Pb concentration and GFR, creatinine clearance, uric acid clearance or uric acid excretion fraction (Karimooy et al., 2010). However, interpretation of this study is limited by the fact that "only 30 subjects had a correct 24 hours urine volume" and no methods are described for kidney outcome measurement or analysis.

Overall, the occupational literature published in the last five years on the kidney impact of Pb exposure has been more consistent in reporting statistically significant associations than were data reviewed for the 2006 Pb AQCD. This may reflect increased reliance on EBE markers as more sensitive outcome measures, publication bias, or multiple comparisons due to a greater number of outcomes assessed. The limitations and lack of consistent results discussed above suggest putting less weight on these occupational studies.

A small number of publications that include concentration-response information provides evidence of Pb-related nephrotoxicity in the occupational setting across the blood Pb ranges analyzed (Weaver et al., 2003a; Ehrlich et al., 1998). Data in 267 Korean Pb workers in the oldest age tertile (mean age = 52 years) did not provide evidence of a threshold for a Pb effect on serum creatinine levels (added variable plot shown in Figure 4-32) (Weaver et al., 2003a). It is important to note the uncertainty regarding whether the concentration-response information provided in these studies applies to lower blood Pb levels or to populations with lower current environmental Pb exposures.



Note: Both the adjusted regression line (straight line) and the line estimated by the smoothing method of the S-PLUS statistical software function lowess (line with curves) are displayed. Both have been adjusted for covariates. For ease of interpretation, axes have been scaled, so that the plotted residuals are centered on the means, rather than zero.

Source: Reprinted with permission of the BMJ Publishing Group, Weaver et al. (2003a)

Figure 4-32 Added variable plot of association between serum creatinine and blood Pb in 267 Korean Pb workers in the oldest age tertile.

A major challenge in interpretation of the occupational literature is the potential for Pb-related hyperfiltration. Hyperfiltration involves an initial increase in glomerular hypertension which results in increased GFR. If persistent, the risk for subsequent CKD increases. This pattern has been observed in diabetes, hypertension, and obesity (Nenov et al., 2000). As discussed in the 2006 Pb AQCD (U.S. EPA, 2006b), findings consistent with hyperfiltration have been observed in occupational populations (Weaver et al., 2003a; Hsiao et al., 2001; Roels et al., 1994), a study of adults who were Pb poisoned as children (Hu, 1991), and a study in European children (de Burbure et al., 2006). Longitudinal data in Pb-exposed rodents provide evidence of a hyperfiltration pattern of increased, followed by decreased GFR, associated with Pb exposure and are critical in interpretation of the human Pb-kidney literature (Khalil-Manesh et al., 1992b). Pb could induce glomerular hypertension resulting in hyperfiltration by several mechanisms including increased ROS, changes in eicosanoid levels, and/or an impact on the renin-

angiotensin system (Vaziri, 2008b; Roels et al., 1994). Whether hyperfiltration contributes to pathology in humans is unclear; longitudinal studies are needed.

The 2006 Pb AQCD provided several explanations for this inconsistency (U.S. EPA, 2006b) (Chapter 6, pp 99):

> "Some are unique to the occupational literature, such as smaller sample sizes. In addition, employed workers are typically healthier and younger than the general population—resulting in the healthy worker bias. This is a particular problem as susceptible risk groups are identified. Survivor bias in cross-sectional studies is also a concern, since workers whose renal function has declined are generally removed from exposure, particularly if they are followed in a medical surveillance program. Few studies have included former workers. Also, statistical analyses have been more limited in occupational studies. Analyses for some outcomes were limited to comparisons between exposed workers and controls whose Pb levels were in the range associated with adverse renal outcomes in environmental work. Use of multiple linear regression has generally involved more limited adjustment for covariates than in most of the environmental studies. Many of these limitations result in bias towards the null, which increases the risk that true associations may not be detected."

Regardless, significant findings could be obscured if opposite direction associations are present in different segments of the study population and interaction models are not performed to address this. In the Korean Pb workers (Weaver et al., 2003a; Weaver et al., 2003b), significant associations in opposite directions were observed only when relevant effect modifiers such as age or genetic variants in ALAD, VDR, and NOS were included in the model. This is a valid concern for risk assessment, since the factors involved in these inverse associations in Pb-exposed workers are not well defined at present.

4.5.2.2 Epidemiology in Children

Pb Nephrotoxicity in Children

Both the 2006 and 1986 Pb AQCDs noted that the degree of kidney pathology observed in adult survivors of untreated childhood Pb poisoning in the Queensland, Australia epidemic (Inglis et al., 1978) has not been observed in other studies of childhood Pb poisoning. Recent publications remain consistent with that conclusion; a recent study observed an impact of childhood Pb poisoning on IQ but not kidney outcomes (Coria et al., 2009). Chelation was raised as a potential explanation for this discrepancy in the 2006 Pb AQCD.

With declining Pb exposure levels, recent work has focused on studies in children with much lower blood Pb levels. However, clinical kidney outcome (i.e., GFR) measures for early kidney damage are insensitive in children who do not have many of the other kidney risk factors that adults do, such as hypertension and diabetes. As a result, such

studies have utilized EBE markers. However, data to determine the predictive value of such biomarkers for subsequent kidney function decline in Pb exposed populations are extremely limited (Coratelli et al., 1988) and may pose particular challenges in children due to puberty-related biomarker changes (Sarasua et al., 2003). The few studies included in the 2006 Pb AQCD (U.S. EPA, 2006b) that analyzed clinical kidney outcomes in children found associations with indicators of Pb exposure that were inconsistent in direction. Fels et al. (1998) found no difference in mean serum creatinine between 62 children living near Pb-producing factories and 50 control children living in communities without Pb emission sources. No significant associations between blood Pb level and kidney function were observed in a population of adults (described in Section 4.5.2.1) and children (N = 200) living around smelters in France; however, beta coefficients were not reported (De Burbure et al., 2003). This study was expanded to include data from 300-600 European children (N varied by outcome) and reported higher concurrent blood Pb level associated with lower serum creatinine and cystatin C (de Burbure et al., 2006). Conversely, in a study of 200 Belgian adolescents aged 17 years, higher concurrent blood Pb level was associated with higher serum cystatin-C (Staessen et al., 2001).

Recent studies of children with elevated Pb exposure did not consistently indicate that Pb exposure was associated with reduced kidney function. A study in 123 children of workers in Pakistani Pb smelters and battery recycling plants and 123 control children, ages 1-6 years, reported elevated blood Pb levels, serum creatinine and urea in children of Pb-exposed workers compared to controls (medians: 8.1 versus 6.7 µg/dL; 56 versus 52 µM; and 4,500 versus 4,300 µM, respectively ($p \leq 0.01$ for all) in unadjusted analyses (Khan et al., 2010a). Blood Pb levels were correlated with serum creatinine (Spearman $r = 0.13$; $p = 0.05$). However, a study of 77 participants, ages 10-25 years, who were previously Pb poisoned through contaminated flour and chelated, reported no difference in renal effects between children with blood Pb levels >48 µg/dL and <43 µg/dL although lower IQ was observed in the subset who were exposed before the age of six years (Coria et al., 2009).

One of the key gaps identified in the 2006 Pb AQCD (U.S. EPA, 2006b) was limited data in children and adolescents particularly with respect to GFR measures and in populations without the elevated Pb exposure associated with Pb poisoning, living near a Pb source, or having parents with occupational Pb exposures. A recently published NHANES analysis in adolescents begins to fill this gap (Fadrowski et al., 2010). Associations between concurrent blood Pb and kidney function were investigated in 769 adolescents aged 12-20 years in the U.S. NHANES III, conducted 1988-1994. Kidney function was assessed with two eGFR equations. One utilized serum cystatin C and the other used the more traditional marker, serum creatinine. Median concurrent blood Pb and cystatin C-based eGFR levels were 1.5 µg/dL and 112.9 mL/min/1.73 m^2, respectively. Cystatin C-

based eGFR was lower (-6.6 mL/min/1.73 m^2 [95% CI: -0.7, -12.6]) in participants with blood Pb levels in the highest quartile (≥ 3.0 µg/dL) compared with those in the lowest (<1 µg/dL). A doubling of blood Pb level was associated with a -2.9 mL/min/1.73 m^2 (95% CI: -0.7, -5.0) lower eGFR. In contrast, the association between blood Pb and creatinine-based eGFR, although in the same direction, was not statistically significant. As these children were born between 1968 and 1982, some likely had higher Pb exposures in earlier childhood, although notably, not as high or as long in duration as did older adults examined in aforementioned studies. Nonetheless, in this study of NHANES adolescents, there also is uncertainty regarding the magnitude, timing, frequency, and duration of Pb exposure that contributed to the observed associations. Additional research in children is warranted, in particular studies with longitudinal follow-up, multiple outcome assessment methods, and examination of children born after Pb was banned from gasoline.

4.5.2.3 Associations between Pb Dose and New Kidney Outcome Measures

As noted above, in an effort to more accurately estimate kidney outcomes, new equations to estimate GFR based on serum creatinine have been developed, and the utility of other biomarkers, such as cystatin C, as well as equations based on them, are being studied. However, few publications have utilized these state-of-the-art techniques when evaluating associations between Pb or Cd dose and renal function. In addition to the study in NHANES adolescents discussed above (Fadrowski et al., 2010), a cross-sectional study of Swedish women reported that higher concurrent blood Pb (median: 2.2 µg/dL) and Cd (median: 0.38 µg/L) levels were associated with lower eGFR based on serum cystatin C alone (without age, sex, and race) after adjustment for socio-demographic and CKD risk factors (Akesson et al., 2005). Associations were comparable to those using creatinine clearance as the kidney outcome for Pb; however associations of Cd dose measures were stronger for the cystatin C based outcome. Staessen et al. (2001) found a statistically significant association between concurrent blood Pb level and serum cystatin C in a cross-sectional study of adolescents; creatinine-based measures were not reported. However, in a cross-sectional study of 804 European children aged range 8.5 to 12.3 years, higher concurrent blood Pb levels were associated with lower serum cystatin C and creatinine; these inverse associations were attributed to hyperfiltration (de Burbure et al., 2006). A recent publication compared associations of blood Pb and eGFR using the traditional MDRD equation to those with four new equations: CKD-EPI, and cystatin C single variable, multivariable, and combined creatinine/cystatin C, in 3,941 adults who participated in the 1999-2002 NHANES cystatin C subsample (Spector et al., 2011). Similar to the NHANES adolescent analysis, associations with the cystatin C outcomes

were stronger. After multivariable adjustment, differences in mean eGFR for a doubling blood Pb were -1.9 (95% CI: -3.2, -0.7), -1.7 (95% CI: -3.0, -0.5), and -1.4 (95% CI: -2.3, -0.5) mL/min/1.73 m^2, using the cystatin C single variable, multivariable and combined creatinine/cystatin C equations, respectively, reflecting lower eGFR with increased blood Pb. The corresponding differences were -0.9 (95% CI: -1.9, 0.02) and -0.9 (95% CI: -1.8, 0.01) using the creatinine-based CKD-EPI and MDRD equations, respectively.

4.5.2.4 Reverse Causality

As noted earlier, the reverse causality hypothesis suggests that the observed associations between blood Pb and kidney function may be due to reduced excretion of Pb associated with prior kidney damage rather than a causal association between Pb exposure and this outcome. The potential for a bidirectional relationship because of reverse causality is plausible in observational epidemiologic studies.

There are several techniques that can be used to assess the potential for reverse causality to underlie associations between higher Pb dose and worse kidney function. Prospective studies in which there are associations between baseline measurements of Pb biomarkers and subsequent changes in renal function provide evidence to evaluate the possibility of reverse causality. In the NAS, baseline blood Pb levels were associated with subsequent declines in renal function over follow-up periods ranging from 3 to 6 years (Tsaih et al., 2004; Kim et al., 1996). Prospective data in CKD patients also revealed an association between baseline Pb dose and decline in eGFR over follow-up periods as long as four years (Yu et al., 2004). However, initial stages of renal decline are often difficult to measure using clinical tests and baseline Pb levels may be accompanied by initial stages of renal function decline. Another approach involves sensitivity analyses in which associations are explored in participants with normal glomerular filtration. This approach has been used in the analyses of the NAS; plots revealed that the association between blood Pb level and serum creatinine was present across the entire range of serum creatinine levels, including those in the normal range where excretion is not impaired (Tsaih et al., 2004; Kim et al., 1996). Analyses restricted to the population with serum creatinine below 1.5 mg/dL were conducted in a later publication and the authors reported that associations were robust (Tsaih et al., 2004). The use of serum creatinine rather than eGFR to establish a normal kidney function cut-off is a limitation since there can be substantial decrements in renal function with ‘normal’ serum creatinine. The associations observed in both NAS studies were not limited to the segment of the population with potentially clinically significant renal dysfunction in whom reduced Pb excretion would be more likely.

Additional evidence evaluating reverse causality was provided by findings among 153 adults with chronic kidney disease, in which renal failure was not associated with increases in blood or bone Pb levels or chelatable Pb levels (Van de Vyver et al., 1988). Batuman et al. (1983) found that chelatable Pb levels were similar in 27 adults with renal disease of unknown and known non Pb-related causes where bone Pb levels (group means: 18 and 19 µg/g) were in the range of those measured in recent epidemiologic studies. A pilot study of 55 ESRD cases at Tulane clinics (Muntner et al., 2007) reported the median blood Pb level was significantly higher among the ESRD cases compared to their control counterparts. For ESRD patients the distribution of blood Pb was shown by pre-defined levels: 18.5% less than 5 µg/dL; 66.7% of ESRD cases had blood Pb levels between 5 and 9.9 µg/dL; and 14.8% equal or greater 10 µg/dL. Other studies have reported elevated blood and bone Pb concentrations in patients with compromised renal function, but prior elevated Pb exposure was often present. Hemodialysis patients exposed to Pb in water (Davenport et al., 2009) and exposed occupationally or to alkyl Pb from atmospheric contamination from gasoline additives (Colleoni et al., 1993) had higher blood Pb level compared to controls. Behringer et al. (1986) reported greater blood Pb levels in patients with renal failure, but points to prior Pb exposure. Winterberg et al. (1990) noted that bone Pb measured in hemodialysis patients without known Pb exposure was elevated compared to chronic renal failure patients, patients after renal transplantation, and a control group.

Although reverse causality has been typically considered plausible in the interpretation of studies of CKD patients whose kidneys are known to be compromised, the reverse causality hypothesis can be broadened to include a physiologic process in which Pb levels are influenced over the entire range of kidney filtration rates. If this occurs, even normal kidney function would impact blood Pb levels such that higher GFR would result in greater Pb excretion and lower blood Pb levels. Serum creatinine levels are influenced in this way over the entire range of kidney function; as a result, these levels are used to estimate kidney function. However, creatinine is produced and excreted at a steady state in the body which is one reason it was selected as a biomarker to assess kidney function. This expanded hypothesis implies that low blood and bone Pb levels may reflect kidney function in addition to exposure. If so, this would increase misclassification bias, with Pb biomarkers reflecting both exposure and kidney function. Given the longstanding use of blood Pb as a dose marker in research for many non-kidney outcomes this seems unlikely. Thus, published research has not directly addressed this. One such approach involves comparing associations of blood and urine Pb in models of kidney function. If they are consistent, this hypothesis is not valid. However, urine Pb is rarely used and may not be as reliable a biomarker as blood Pb (Gulson et al., 1998c).

In summary, several lines of evidence support that reverse causality does not contribute substantially to associations between higher blood Pb levels and worse kidney function. These lines of evidence include prospective data observing that baseline Pb measures are associated with subsequent declines in renal function, that associations in prospective studies persist among adults with normal renal function, and that some studies indicate renal failure does not increase Pb biomarker levels. Reverse causation remains a completely plausible hypothesis. The existence of an association in adults with normal renal function does not exclude reverse causation because it is not known how Pb clearance varies within the range of normal kidney function. Thus, this bidirectional relationship is possible and additional evidence is needed to fully elucidate the extent to which diminished kidney function may itself result in increased Pb levels.

4.5.2.5 Toxicology

In animals, Pb has been found to induce changes in a wide range of indicators of renal function. Most studies examined Pb exposure concentrations that resulted in higher blood Pb levels (> 20 µg/dL) than those in the current U.S. general population. While toxicological information on renal dysfunction with blood Pb levels < 20 µg/dL generally is not available, dysfunction in kidney function measures, including urinary flow, ALP, microalbumin, and NAG, was observed at blood Pb levels above 20 µg/dL.

Table 4-28 Animal toxicological studies reporting the effects of Pb exposure (as blood Pb level) on kidney function.

Reference	Species; Lifestage; Sex	Pb Dose; Exposure Duration	Blood Pb Level with Response (µg/dL)	Responses
Berrahal et al. (2011)	Rat; Adult	50 ppm Pb acetate in drinking water; lactation day 1 to PND40 or PND65	PND40: 12.7 PND65: 7.5	Oxidative stress – Increased renal MDA (PND40 and PND65), decreased renal SOD activity (PND40) Morphology – Increased relative kidney weight (PND65) Kidney function – Increased blood creatinine (PND40 and PND65), increased BUN (PND40), decreased uric acid (PND65), increased kidney proteins (PND65).
Massó-González et al. (2009)	Rat; Weanling pups	300 ppm Pb acetate in drinking water; GD1 to PND21	23	Oxidative stress – Elevated TBARS and catalase activity Morphology – Elevated relative kidney weight at PND21
Roncal et al. (2007)	Rat; Adult; Male	150 ppm Pb acetate in drinking water; 16 weeks with remnant kidney surgery at week 4	26	Inflammation – Increased the number of macrophages & renal MCP-1 mRNA. Morphology – Pb induced pre-glomerular vascular disease of kidney (i.e., sclerosis, fibrosis, peritubular capillary loss) Kidney function – Decreased creatinine clearance, increased serum creatinine, increased BUN, and increased serum uric acid.
Khalil-Manesh et al. (1993a)	Rat; Adult; Male	100 ppm Pb acetate in drinking water; 12 months	Mean at 3 months 29.4 Mean at 12 months 22 Range 9-34	Morphology – Mild tubular atrophy and interstitial fibrosis seen at 12 months, otherwise normal. Kidney function – Increased GFR at 1 and 3 months, increased NAG, and no change in GST.
Khalil-Manesh et al. (1992a)	Rat; Adult; Male	5,000 ppm Pb acetate in drinking water; 1, 6, or 9 months	At 1 month 7.9 At 6 months ~30 At 9 months 52	At 1 month Kidney function – no functional or pathological changes At 6 months Morphology – Prominent tubulointerstitial fibrosis and segmental sclerosis. Increased kidney weight Kidney function – Increased serum creatinine and SUN, no change urinary NAG or GST At 9 months Morphology – Severe tubulointerstitial disease Kidney function – Decreased GFR, increased serum creatinine and SUN
Vyskocil et al. (1995)	Rat, Adult, Female	1,000 ppm Pb acetate in drinking water; 2 or 4 months	37.6	Kidney function – No change in kidney function or nephrotoxicity

Table 4-28 (Continued): Animal toxicological studies reporting the effects of Pb exposure (as blood Pb level) on kidney function.

Reference	Species; Lifestage; Sex	Pb Dose; Exposure Duration	Blood Pb Level with Response (µg/dL)	Responses
Ademuyiwa et al. (2009)	Rat; Adult	200, 300, and 400 ppm Pb acetate in drinking water; 12 weeks	200 ppm: 41 300 ppm: 61 400 ppm: 39	Kidney function – Renal phospholipidosis and depletion of renal cholesterol.
Navarro-Moreno et al. (2009)	Rat; Adult; Male	500 ppm Pb acetate in drinking water; 28 weeks	43	Oxidative stress – Increased kidney lipid peroxidation (i.e., TBARS) Morphology – Electron micrography showed lumen reduction, microvilli loss, brush border loss, and mitochondrial damage Kidney function – Elevated urinary pH and protein, and glucose and blood in the urine.
Khalil-Manesh et al. (1992b)	Rat; Adult; Male	5,000 ppm Pb acetate in drinking water; 12 months	Max 125 Mean 45	Morphology – Tubular atrophy and interstitial fibrosis after 6 months. Increased urinary brush border antigens. Kidney function – Hyperfiltration at 3 months and decreased GFR at 12 months. After 3 months, elevated urinary NAG and GST.
Wang et al. (2010e)	Rat; Adult; Female	300 ppm Pb acetate in drinking water; 8 weeks	20 (serum)	Biomarker – Aberrant NAG, GGT, β2-microgobulin expression Oxidative Stress – Increased lipid peroxidation (i.e., MDA production), elevated kidney antioxidant enzymes (SOD, GPx, CAT), and depleted GSH Morphology – Electron micrography showed Pb damages mitochondria, basement membrane, and brush border in kidney tissue. Some focal tubal necrosis observed. Kidney function – Elevated urinary total protein, urinary albumin, and serum urea nitrogen.

Renal Function and Interstitial Fibrosis

Past studies have shown that chronic continuous or repeated Pb exposure can result in interstitial nephritis and focal or tubular atrophy. A series of studies on Pb exposure in rats (longitudinal 12-month exposure study to either 100 ppm or 5,000 ppm Pb in drinking water) report an initially elevated GFR, consistent with hyperfiltration, and renal hypertrophy (Khalil-Manesh et al., 1993a; Khalil-Manesh et al., 1992b; Khalil-Manesh et al., 1992a). After 6 months of exposure, GFR decreased, albuminuria was present, and pathology ensued with focal tubular atrophy and interstitial fibrosis formation. This pathology and functional decrement was persistent out to 12 months, and at 12 months glomeruli developed focal and segmental sclerosis.

The toxicological evidence for differences in GFR according to duration of Pb exposure (i.e., hyperfiltration with 3-month exposure versus decreased GFR with 6- or 12-month exposure) provides biological plausibility for epidemiological studies that observed a similar phenomenon by age in adults in association with Pb biomarker levels. However, the toxicological studies report blood Pb levels at some exposure durations much higher than relevant to blood Pb levels in current human populations. Still, these duration-dependent dichotomous changes in GFR are consistent between the toxicological and epidemiologic literature.

At exposure concentrations resulting in blood Pb levels within one order of magnitude of the upper range of current human population blood Pb level (< 30 µg/dL), animal toxicological studies present inconsistent results for the effects of Pb on kidney function (Table 4-28). There are studies that have corroborated the previously observed increase in serum creatinine following Pb exposure in rats. Berrahal et al. (2011) reported on the effects of age-dependent exposure to Pb on nephrotoxicity in male rats (Table 4-29). Pups were exposed to Pb lactationally (as a result of dams consuming water containing 50 ppm Pb acetate) until weaning. Thereafter, the offspring were exposed to the same solution from weaning (day 21) until sacrifice. Male pups were sacrificed at age 40 days (puberty; blood Pb level 12.7 µg/dL) and at age 65 days (post-puberty; blood Pb level 7.5 µg/dL). Serum creatinine was elevated at both 40 days and 65 days (0.54 and 0.60 mg/dL compared to control values of 0.45 mg/dL [p <0.001]). Various parameters of Pb-induced renal dysfunction are listed in Table 4-29. The elevated serum creatinine in the Pb-exposed animals compared to controls suggests that animals exposed to low dose (i.e., 50 ppm) Pb from birth may develop renal abnormalities. However, the lack of measurements of GFR or renal pathology weakens the conclusions.

Table 4-29 Indicators of renal damage in male rats exposed to 50 ppm Pb for 40 and 65 days, starting at parturition.

Biomarker (Mean ± SD)	PND40 Control	PND40 Pb	PND65 Control	PND65 Pb
Blood Pb level (µg/dL)	1.8 ± 0.33	12.7 ± 1.7	2.1 ±n0.35	7.5n± 0.78
Plasma Creatinine (mg/L)	4.5 ± 0.21	5.35 ± 0.25[a]	4.55n± 0.27	6.04 ± 0.29[a]
Plasma Urea (mg/L)	0.37 ± 0.019	0.47 ± 0.021[a]	0.29n± 0.009	0.29n± 0.009
Plasma Uric Acid (mg/L)	7.51 ± 0.44	7.65 ± 0.32	9.39n± 0.82	5.91n± 0.53[a]

[a]p <0.001

Source: Modified with permission of John Wiley & Sons, Berrahal et al. (2011)

Roncal et al. (2007) found that Pb accelerated renal function decrements, tubulointerstitial injury, and arteriolopathy in non-Pb-related CKD. Sprague-Dawley rats were administered Pb acetate at 150 ppm for 4 weeks, then subjected to remnant kidney surgery (left kidney mass reduced by 2/3 and right kidney removed), and subsequently exposed to Pb for an additional 12 weeks resulting in a blood Pb level of 26 µg/dL. Pb-treated rats had higher systolic BP, increased serum creatinine, lower creatinine clearance, and higher proteinuria than did controls. Most striking was development of worse arteriolar disease, peritubular capillary loss, tubulointerstitial damage, and macrophage infiltration. Pb treatment was associated with significant worsening of pre-glomerular vascular disease, as characterized by an increase in the media-to-lumen ratio. There was also a higher percentage of segmental sclerosis within glomeruli and a tendency for a higher number of sclerotic glomeruli. Additionally, a loss of peritubular capillaries, as reflected by a reduction in thrombomodulin staining, was observed. This was associated with worse tubular injury (osteopontin staining) due to more interstitial fibrosis (type III collagen staining) and a greater macrophage infiltration in the interstitium. The increase in macrophages was associated with higher renal MCP-1 mRNA. As a whole, these findings indicate that Pb exposure concomitant with existing renal insufficiency due to surgical kidney resection accelerated vascular disease and glomerular pathology. These findings are consistent with the previous work of Bagchi and Preuss (2005) also showing that Pb-exposed animals with non-Pb-related CKD (remnant surgery) had kidney dysfunction including impairment of the renin-angiotensin system (Losartan challenge), elevated systolic BP, and alterations in renal excretion of Pb, K^+, and Na^+. Thus, this model shows that Pb exposure may exacerbate pre-existing underlying kidney disease.

Other investigators have shown that chronic Pb exposure has detrimental effects on renal function at higher blood Pb levels. A number of studies report increased serum creatinine following high level Pb exposure (e.g., blood Pb levels >55.6 µg/dL) (Abdel Moneim et al., 2011b; Ozsoy et al., 2010; Jayakumar et al., 2009; Kharoubi et al., 2008a). In addition, studies reporting high blood Pb levels and high Pb exposure levels report increased urine, serum, or blood urea nitrogen (Wang et al., 2010e; El-Nekeety et al., 2009; Jayakumar et al., 2009; Kharoubi et al., 2008a). Jayakumar et al. (2009) reported alterations in other markers of kidney toxicity, lysosomal marker and brush border enzymes (i.e., ALP, ACP, γ-GT, NAG, β-D-glucuronidase), following Pb exposure (2,000 ppm for 6 weeks). Similarly, Wang et al. (2010e) reported time-related increases in urinary alkaline phosphatase, urinary GGT, urinary NAG, urinary total protein, urinary β-2 microglobulin, and urinary microalbumin following Pb exposure (300 ppm in drinking water, serum Pb level 20 µg/dL). Pb-exposed male rats (500 ppm Pb acetate in drinking water for 7 months, blood Pb level 43 µg/dL) had elevated urinary pH, urinary glucose, and proteinuria (Navarro-Moreno et al., 2009).

Qiao et al. (2006) measured the effect of Pb on the expression of the renal nuclear factor-kappa B (NF-κB), transforming growth factor (TGF-β) and fibronectin in Sprague-Dawley rat kidney. These growth (TGF-β) and transcription (NF-κB) factors modulate the progression of renal function decrements through promotion of extracellular matrix (fibronectin) synthesis and promotion of fibrosis. Pb was administered at a dose of 5,000 ppm Pb acetate, continuously for either one, two, or three months. All factors increased by the end of three months of treatment, but only NF-κB increased progressively at each time period. These changes were hypothetically related to the development of Pb-induced renal fibrosis in rats, but no histology was performed.

The renal effects of chronic Pb exposure as detailed above were partially mitigated in rats following lowering of blood Pb level with chelation therapy (i.e., DMSA) (Khalil-Manesh et al., 1992a) and after treatment with antioxidants (Abdel Moneim et al., 2011b; Ozsoy et al., 2010; Wang et al., 2010e; El-Nekeety et al., 2009; Jayakumar et al., 2009; Kharoubi et al., 2008a). DMSA treatment improved renal function; however, Pb-induced pathology remained (Khalil-Manesh et al., 1992a). Improvements include increased GFR, decreased albuminuria, and decreased inclusion body numbers but little change in tubulointerstitial scarring. DMSA also acts as an antioxidant, so the protective effects may not be entirely attributed to the lowering of blood Pb level. Similarly, several studies found that treatment with antioxidant compounds could protect against Pb-induced kidney dysfunction. Administration of flaxseed oil, L-carnitine, NAC, and several medicinal plants including, *Achyranthes aspera*, *Artemisia absinthium*, and *Aquilegia vulgaris*, to Pb-exposed rodents protected against injury to the kidney or restored kidney function (Abdel Moneim et al., 2011b; Ozsoy et al., 2010; Wang et al., 2010e; El-Nekeety et al., 2009; Jayakumar et al., 2009; Kharoubi et al., 2008a). These studies suggest that a reduction in reactive oxygen species may attenuate the effects of Pb on kidney function implicating oxidative stress as a predominant mechanism for Pb-induced reduced kidney function.

Histological Changes

Earlier studies discussed in previous Pb AQCDs have identified Pb-related renal damage by the presence of dense intranuclear inclusion bodies, which are capable of sequestering Pb (Goyer et al., 1970b). Pb-induced formation of intranuclear inclusion bodies in the proximal tubule (PT) is considered protective; Pb is sequestered such that it is not in its bioavailable, free, toxicologically active form. Intranuclear inclusion bodies are found in the kidney with short-term (i.e., <4 weeks) Pb exposure but present to a lesser degree with chronic exposures (see Section 4.2.3 for further discussion). Chelators such as $CaNa_2EDTA$ have removed these inclusion bodies from affected nuclei (Goyer et al., 1978).

Multiple ultrastructural changes indicate dysfunction in the PT and nephropathy after Pb exposure, including changes to the PT epithelium, endoplasmic reticulum dilation, nuclear membrane blebbing, and autophagosome enlargement (Fowler et al., 1980; Goyer et al., 1970a). Indications similar to the PT transport-associated Fanconi syndrome appear with Pb exposure, albeit often at high doses of Pb, i.e., Pb poisoning. These indications, which include increased urinary electrolyte excretion (Zn), decreased Na^+/K^+ATPase activity, mitochondrial aberrations, and aminoaciduria, also have been associated with blood Pb levels in children.

Recent studies since the 2006 Pb AQCD are consistent with the earlier findings and build upon the literature base by including the role of antioxidants. Jabeen et al. (2010) exposed pregnant albino BALB/c mice to a daily oral dose of Pb acetate (10 mg/kg body weight, daily throughout pregnancy) until GD18, at which point the fetal kidneys were processed for histological examination. Histology revealed Pb exposure induced decreased kidney cortical thickness, decreased diameter of renal corpuscles, and increased renal tubular atrophy (with desquamated epithelium and degenerated nuclei in the distal and proximal tubules). Blood Pb levels were not reported in this study. Nonetheless, these data show that in utero Pb exposure had significant histological effects on the fetal kidney, which could contribute to altered renal function including clearance of waste products, electrolyte balance, and vasoregulation.

Massanyi et al. (2007) reported on Pb-induced alterations in male Wistar rat kidneys after single i.p. doses of Pb acetate (50, 25, and 12.5 mg/kg); kidneys were removed and analyzed 48 hours after Pb administration. Qualitative microscopic analysis detected dilated Bowman's capsules and dilated blood vessels in the interstitium with evident hemorrhagic alterations. Quantitative histomorphometric analysis revealed increased relative volume of interstitium and increased relative volume of tubules in the experimental groups. The diameter of renal corpuscles and the diameter of glomeruli and Bowman's capsule were significantly increased. Measurement of tubular diameter showed dilatation of the tubule with a significant decrease of the height of tubular epithelium compatible with degenerative renal alterations. These findings extend the observations of Fowler et al. (1980) and Khalil-Manesh et al. (1992b; 1992a); in particular, the enlarged glomeruli are consistent with the early hyperfiltration caused by Pb.

Abdel Moneim et al. (2011b) reported histological evidence of inflammation after Pb treatment in rats (i.p. 20 ppm, 5 days). This evidence included increased inflammatory cellular infiltrations, cytoplasmic vacuolation, and dilatation of some kidney tubules. Inflammation was accompanied by an increase in apoptotic cells and increased oxidative stress.

A recent study has also reported inclusion body formation in the nuclei, cytoplasm, and mitochondria of PT cells of Pb-treated rats (50 mg Pb/kg bw, i.p., every 48 hours for 14 days) (Navarro-Moreno et al., 2009). These inclusion bodies were not observed in chronically Pb-exposed rats (500 ppm Pb in drinking water, 7 months). However, chronic Pb exposure resulted in morphological alterations including loss of PT apical membrane brush border, collapse and closure of the PT lumen, and formation of abnormal intercellular junctions.

Vogetseder et al. (2008) examined the proliferative capacity of the renal PT (particularly the S3 segment) following i.v. administration of Pb to juvenile and adult male Wistar rats. Proliferation induction was examined by detection of Bromo-2'-deoxyuridine (BrdU), Ki-67 (labels S, G2, and M phase cells), and cyclin D1 (an essential cell cycle progression protein). The cycling marker Ki-67 revealed a much higher proliferation rate in the S3 segment in control juvenile rats (4.8 ± 0.3%) compared with control adult rats (0.4 ± 0.1%). Pb administration (3.8 mg/100 g bw) increased the proportion of Ki-67-positive cells to 26.1 ± 0.3% in juvenile rats and 31.9 ± 0.3% in adult rats. Thus, the increased proliferation caused by Pb was age independent. The proliferation induction caused by Pb administration may be a result of reduced cell cycle inhibition by $p27^{kip-1}$. Acute Pb treatment increased the incidence of cyclin D1 labeling in the BrdU-positive cells suggesting Pb was able to accelerate re-entry of cells into the cell cycle and cause proliferation in the PT. Pb-induced cellular proliferation has also been reported in the retina with gestational and early postnatal rodent Pb exposure (Giddabasappa et al., 2011).

Ademuyiwa et al. (2009) examined Pb-induced phospholipidosis and cholesterogenesis in rat tissues. Sprague-Dawley rats were exposed to 200, 300, and 400 ppm Pb acetate for 12 weeks. The Pb exposure resulted in induction of phospholipidosis in kidney tissue, accompanied by depletion of renal cholesterol. The authors suggested that induction of cholesterogenesis and phospholipidosis in kidney may be responsible for some of the subtle and insidious cellular effects found with Pb-mediated nephrotoxicity. Drug-induced PT phospholipidosis is seen clinically with use of the potentially nephrotoxic aminoglycoside drugs, including gentamicin (Baronas et al., 2007).

Various antioxidants have been shown to attenuate Pb-induced histopathological changes to the kidney. Ozsoy et al. (2010) found L-carnitine to be protective in a model of experimental Pb toxicity in female rats. Markers of histopathological change in the kidney, including tubule dilatation, degeneration, necrosis, and interstitial inflammation were rescued by L-carnitine treatment in females. Male rats exposed to Pb (2,000 ppm for 6 weeks) also displayed tubular damage, whereas concomitant treatment with Pb and an extract of *Achyranthes aspera* ameliorated the observed damage (Jayakumar et al., 2009).

El-Sokkary et al. (2005) reported Pb-induced (100 ppm, s.c. for 30 days) tubular degeneration with necrotic cells that could be prevented with melatonin treatment. Melatonin is known to be an efficacious free radical scavenger and indirect antioxidant. El-Nekeety et al. (2009) found an extract of the folk medicine plant *Aquilegia vulgaris* to be protective against Pb acetate-induced tubular dilatation, vacuolar and cloudy epithelial cell lining, interstitial inflammatory cell infiltration, hemorrhage, cellular debris, and glomerulus hypercellularity. Concomitant exposure to Pb and extract produced histology indiscernible from that in controls. Post treatment with extract partially rescued the Pb-induced histopathology. El-Neweshy and El-Sayed (2011) studied the influence of vitamin C supplementation (20 mg/kg pretreatment every other day) on histopathological alterations in Pb-exposed male rats (20 mg/kg by intragastric feeding once daily for 60 days). Control rats showed normal histology, while Pb-treated rats exhibited karyomegaly with eosinophilic intranuclear inclusion bodies in the epithelial cells of the proximal tubules. Glomerular damage and tubular necrosis with invading inflammatory cells were also found. Rats treated with Pb acetate plus vitamin C exhibited relatively mild or no karyomegaly with eosinophilic intranuclear inclusion bodies in the proximal tubules. Normal glomeruli were noted in animals exposed to Pb and vitamin C. These findings consistently show that some antioxidants are capable of preventing or rescuing Pb-induced renal histopathological changes, suggesting a role for oxidative stress in the development of Pb-induced nephropathy.

Table 4-30 presents the acute and chronic renal effects of Pb exposure observed in recent and past animal toxicology studies.

Table 4-30 Effects of Pb on the kidney/renal system related to exposure duration: Evidence from animal (rodent) toxicology studies.

Effects with less than 3 months of exposure	Effects with 6 or 12 months of exposure
Mitochondrial dysfunction	Mitochondrial dysfunction
Renal cell apoptosis	Renal cell apoptosis
Nuclear inclusion body formation	Oxidant redox imbalance
Proximal tubule cytomegaly	Altered NO homeostasis
Glomerular hypertrophy	ATPase dysfunction
Increased GFR	Aminoaciduria
	Increased electrolyte excretion
	Elevated blood pressure
	Decreased GFR

4.5.3 Modes of Action for Pb-Induced Nephrotoxicity

4.5.3.1 Oxidative Damage

A role for ROS in the pathogenesis of experimental Pb-induced hypertension and renal disease has been well characterized (Vaziri, 2008a, b; Vaziri and Khan, 2007). The production of oxidative stress following Pb exposure is detailed in respect to modes of action of Pb (Section 4.2.4). Past studies have shown that Pb treatment (single or three daily i.p. injections) can elevate kidney GST levels, affecting glutathione metabolism (Daggett et al., 1998; Moser et al., 1995; Oberley et al., 1995).

Animal studies continue to provide evidence for increased oxidative stress playing a role in the pathogenesis of Pb-induced renal toxicity. Increased ROS, serum NO, and renal NO were observed after Pb injections in rats (i.p. 20 mg/kg, 5 days) (Abdel Moneim et al., 2011b). Pb exposure to rat proximal tubular cells (0.5-1 µM) also increased ROS production, in a concentration-dependent manner (Wang et al., 2011b). Increased lipid peroxidation (i.e., MDA) was demonstrated in serum and renal tissue after Pb exposure (Abdel Moneim et al., 2011b; Lodi et al., 2011; Wang et al., 2011b). Berrahal et al. (2011) reported increased MDA in the kidney of Pb-exposed (50 ppm Pb acetate pre- and post-natally) rats relative to controls at both 40 (puberty; blood Pb 12.7 µg/dL) and 65 (post-puberty; blood Pb 7.5 µg/dL) days of age. In addition, total sulfhydryl groups were significantly decreased at 65 days. These increases in oxidative stress were accompanied by age-dependent Pb nephrotoxicity in male rats.

Alterations in endogenous antioxidants and antioxidant enzymes that may lead to oxidative stress have also been reported after Pb exposure. Pb treatment decreased the activity of the renal antioxidant enzymes, CAT, SOD, GST, GPx, and GR (Abdel Moneim et al., 2011b) and protein levels of CAT and GSH (Lodi et al., 2011). Additionally, proteomic analysis of high-level Pb treated (1,500 ppm, 5 weeks; resulting in blood Pb level of 53.4 µg/dL) rat kidney identified decreased abundance of a rate-limiting enzyme in the synthesis of GSH (glutamate cysteine ligase) (Chen et al., 2011b).

Conterato et al. (2007) examined the effect of Pb acetate on the cytosolic thioredoxin reductase activity and oxidative stress parameters in rat kidneys. A single injection of Pb acetate consisted of a single i.p. injection of 25 or 50 mg/kg Pb acetate, while repeated injections consisted of one daily i.p. injection of Pb acetate (5 or 25 mg/kg) for 30 days. Measured were thioredoxin reductase-1, a selenoprotein involved in many cellular redox processes, SOD, δ-ALAD, GST, GPx, non protein thiol groups (NPSH), CAT, as well as plasma creatinine, uric acid, and inorganic phosphate levels. The single injection at the 25 mg Pb dose level resulted in increased SOD and thioredoxin reductase-1 activity,

while the 50 mg dose level increased CAT activity and inhibited δ-ALAD activity in the kidney. Repeated injections at the 5 mg dose level of Pb inhibited δ-ALAD and increased GST, NPSH, CAT, and thioredoxin reductase-1. Repeated injections at the 25-mg dose level reduced δ-ALAD but increased GST, NPSH, and plasma uric acid levels. No changes were observed in TBARS, GPx, creatinine or inorganic phosphate levels after either single or repeated injection dosing. As both dosing regimens increased thioredoxin reductase-1 activity, the authors suggest that this enzyme may be a sensitive indicator of renal changes with low dose Pb treatment.

Jurczuk et al. (2006) published a study of the involvement of some low molecular weight thiols in the peroxidative mechanisms of action of Pb in the rat kidney. Wistar rats were fed a diet containing 500 ppm Pb acetate for a period of 12 weeks and were compared to a control group receiving distilled water for the same time period. GSH, metallothionein (MT), total and nonprotein SH groups (TSH and NPSH) were measured, as were the blood activity and urinary concentration of δ-ALA. The concentrations of GSH and NPSH were decreased by Pb administration, while MT concentration was unchanged. δ-ALAD in blood was decreased, whereas urinary δ-ALA was increased by Pb administration. Negative correlations were found between the kidney GSH concentrations and previously reported concentrations of Pb and MDA in kidneys of these rats. It is apparent from graphical presentation of the data that GSH was reduced by more than 50% following Pb administration, while TSH was reduced by approximately 15%. No values for either blood or kidney Pb levels or kidney MDA were reported in this article. In 2007, the same authors (Jurczuk et al., 2007) reported on the renal concentrations of the antioxidants, vitamins C and E, in the kidneys of the same Pb-treated and control rats. Exposure to Pb significantly decreased vitamin E concentration by 13% and vitamin C concentration by 26%. The kidney concentration of vitamin C negatively correlated with MDA concentration. The authors concluded that vitamins E and C were involved in the mechanism of peroxidative action of Pb in the kidney, and their protective effect may be related to scavenging of free radicals.

Studies have used antioxidant compounds to investigate the role of oxidative stress in Pb-induced nephrotoxicity. Abdel Moneim et al. (2011b) reported that flaxseed oil treatment protected rats from Pb-induced (i.p. 20 mg/kg, 5 days) oxidative stress, inflammation, and apoptosis. However, the flaxseed oil also decreased the accumulation of Pb in renal tissue making it difficult to ascertain whether the protection was due to decreased oxidative stress or to altered Pb uptake kinetics.

El-Neweshy and El-Sayed (2011) studied the influence of vitamin C supplementation on Pb-induced histopathological alterations in male rats. Rats were given Pb acetate, 20 mg/kg by intragastric feeding once daily for 60 days. Control rats were given 15 mg of

sodium acetate per kg once daily, and an additional group was given Pb acetate plus vitamin C (20 mg/kg every other day) 30 minutes before Pb feeding. Control rats showed normal histology, while Pb-treated rats exhibited karyomegaly with eosinophilic intranuclear inclusion bodies in the epithelial cells of the proximal tubules. Glomerular damage and tubular necrosis with invading inflammatory cells were also seen in Pb-treated animals. Among rats treated with Pb acetate plus vitamin C, five exhibited relatively mild karyomegaly and eosinophilic intranuclear inclusion bodies of proximal tubules and an additional five rats were normal. Normal glomeruli were noted in all. Thus, vitamin C was shown to ameliorate the renal histopathological effects of Pb intoxication, however no measures of Pb accumulation were provided to clarify the mechanism of action of vitamin C.

Masso-Gonzalez and Antonio-Garcia (2009) studied the protective effect of natural antioxidants (Zn, vitamin A, vitamin C, vitamin E, and vitamin B6) against Pb-induced damage during pregnancy and lactation in rat pups. At weaning, pups were sacrificed and kidneys were analyzed. Pb-exposed pups had decreased body weights. Blood Pb levels were 1.43 μg/dL in the control group, 22.8 μg/dL in the Pb group, 21.2 μg/dL in the Pb plus Zn plus vitamins group, and 0.98 μg/dL in the Zn plus vitamin group. The kidney TBARS were significantly elevated in Pb exposed pups, while treatment with vitamins and Zn returned TBARS to control levels. Kidney CAT activity was significantly increased above control with Pb treatment; however supplementation with Zn and vitamins reduced CAT activity toward normal. Pb exposure inhibited kidney Mn-dependent SOD but not Cu-Zn-dependent SOD activity. Thus, supplementation with Zn and vitamins during gestation and lactation was effective in attenuating the redox imbalance induced by developmental, chronic low-level Pb exposure.

Bravo et al. (2007) reported further that mycophenolate mofetil (an immunosuppressive agent used in renal transplantation which inhibits T and B cell proliferation) administration reduced renal inflammation, oxidative stress and hypertension in Pb-exposed rats. Thus, an inflammatory immune and oxidative stress component can be seen as contributing to Pb-induced renal effects and hypertension.

Although the majority of studies of the effects of Pb exposure have been conducted in male rats, a couple of studies have compared the response of male rats with female rats (Sobekova et al., 2009; Alghazal et al., 2008a). Sobekova et al. (2009) contrasted the activity response to Pb on the antioxidant enzymes, GPx and GR, and on TBARS in both male and female Wistar rats of equal age. Males weighing 412 ± 47 g and females weighing 290 ± 19 g were fed diets containing either 100 ppm or 1,000 ppm Pb acetate for 18 weeks. In the male rats, kidney Pb content increased by 492% on the 100 ppm Pb diet and by 7,000% on the 1,000 ppm Pb diet. In the female rats, kidney Pb content

increased by 410% on the 100 ppm Pb diet and by 23,000% on the 1,000 ppm Pb diet. There was virtually no change in GPx in the kidney of male rats given the 100 ppm Pb diet but there was a significant reduction in GPx in the female rats on both the 100 ppm diet and 1,000 ppm diet. In male rats, GR was increased from 182 units/gram of protein in control kidneys to 220 units on the 100 ppm Pb diet and 350 units on the 1,000 ppm diet. In female rats, kidney GR decreased from 242 units in control animals to 164 units in animals on the 100 ppm Pb diet and 190 units in animals on the 1,000 ppm diet. In male rats, kidney TBARS content increased from 7.5 units/gram protein to 10.0 units (1,000 ppm Pb diet group). In female rats, there was a reduction in TBARS from 14.4 units per gram protein to 10.0 units in rats on the 100 ppm Pb diet and to 11 units in rats on the 1,000 ppm Pb diet.

Alghazal et al. (2008a) compared the activity responses of the antioxidant enzyme, SOD and the detoxifying enzyme, GST, of the same rats exposed to 100 ppm or 1,000 ppm Pb acetate for 18 weeks. Similar to the previous study, kidney TBARS were increased only in male rats given the higher dose of Pb. Kidney SOD activity, on the other hand, was increased in both males and females at the higher dose of Pb, while GST activity was increased in kidney of males at the higher dose of Pb and decreased at the lower dose, but was decreased at both doses of Pb in females. Thus there were significant differences in the responses of male and female rats to Pb exposure. Differences may be accounted for in part due to the greater deposition of Pb in female rat kidneys. Another explanation, offered by the authors, is that male rats are known to metabolize some foreign compounds faster than do females, so the biological half-life of xenobiotics in the females may be longer.

4.5.3.2 Hypertension and Alteration of Renal Vasculature and Reactivity

As discussed in Section 4.5.1, changes in renal vasculature function or induction of hypertension can contribute to further renal dysfunction. Pb exposure increases BP, resulting in hypertension, through the promotion of oxidative stress and altered vascular reactivity (Section 4.4). Antioxidants attenuated Pb-related oxidative/nitrosative stress in the kidney and abrogated the Pb-induced increased BP (Vaziri et al., 1999a). Chronic increases in vascular pressure can contribute to glomerular and renal vasculature injury, which can lead to progressive renal dysfunction and kidney failure. In this manner, Pb-induced hypertension has been noted as one contributer to Pb-induced renal disease.

Also, Pb has been shown to act on known vasomodulating systems in the kidney. In the kidney, two vascular tone mediators, NO and ET-1, are found to be affected by Pb

exposure. Administration of the vasoconstrictor endothelin-1 (ET-1) affected mean arterial pressure (MAP) and decreased GFR (Novak and Banks, 1995). Acute high-dose Pb exposure (24 nmol/min, for 15 or 30 minutes) completely blocked this ET-1-mediated GFR decrease but had no effect on MAP. Depletion of the endogenous antioxidant glutathione using the drug buthionine sulfoximine, a GSH synthase inhibitor, increased BP and increased kidney nitrotyrosine formation without Pb exposure, demonstrating the importance of GSH in maintenance of BP (Vaziri et al., 2000). Multiple studies have shown that Pb exposure depletes GSH stores. Catecholamines are vascular moderators that are also affected by Pb exposure (Carmignani et al., 2000). The effect on BP with Pb exposure is especially relevant to the kidney because it is both a target of Pb deposition and a mitigator of BP. These earlier data detail the interaction of known modulators of vascular tone with Pb.

The renin-angiotensin-aldosterone system (RAAS) plays an important role in kidney homeostasis and alteration of this pathway may affect renal function. A discussion of the RAAS can also be found in Section 4.4.2.3 in relation to the role of RAAS in Pb-induced hypertension. A meta-analysis found that Pb exposure (resulting in blood Pb levels: 30-40 µg/dL) increased plasma renin activity and renal tissue renin in young but not old rats (Vander, 1988). Exposure of experimental animals to Pb also induced increases in plasma, aorta, heart, and kidney angiotensin converting enzyme (ACE) activity; plasma kininase II, kininase I, and kallikrein activities; and renal AngII positive cells (Rodriguez-Iturbe et al., 2005; Sharifi et al., 2004; Carmignani et al., 1999). Simões et al. (2011) reported that acute Pb treatment (Pb acetate i.v. bolus dose of 320 µg/kg bw, blood Pb of 37 µg/dL at 120 minutes after Pb administration) in adult male Wistar rats increased serum angiotensin converting enzyme (ACE) activity. Systolic arterial pressure, but not diastolic arterial pressure or heart rate, was also elevated 60 minutes after treatment. The Pb-induced altered systolic BP attenuated in animals co-treated with Losartan (Ang II receptor blocker) or Enalapril (ACE inhibitor), suggesting a regulatory role for the RAAS (Simões et al., 2011). These data agree with earlier reports of Pb-related increases in ACE activity in young rats exposed to Pb for 2-8 weeks (Sharifi et al., 2004) and adult rats exposed to Pb for 10 months (Carmignani et al., 1999). Lower level Pb (100 ppm, 14 weeks; range of blood Pb levels: 23.7-27 µg/dL) exposure increased renal cortical AngII content and the number of tubulointerstitial AngII-positive cells (Bravo et al., 2007). This heightened intrarenal angiotensin corresponded with sodium retention and increased systolic BP and was ablated by the anti-inflammatory antioxidant, MMF.

Recently, Vargas-Robles et al. (2007) examined the effect of Pb exposure (100 ppm Pb acetate for 12 weeks) on BP and angiotensin II vasoconstriction in isolated perfused kidney and interlobar arteries. Vascular reactivity was evaluated in the presence and absence of the nitric oxide synthase inhibitor L-NAME in both Pb-treated and control

animals. Pb exposure significantly increased BP (134 ± 3 versus 100 ± 6 mmHg), eNOS protein expression, oxidative stress, and vascular reactivity to angiotensin II. L-NAME potentiated the vascular response to angiotensin II in the control group, but had no effect on the Pb-treated group. Conversely, passive microvessel distensibility, measured after deactivation of myogenic tone by papaverine, was significantly lower in the arteries of Pb-exposed rats. Nitrites released from the kidney under the influence of angiotensin II in the Pb group were lower as compared to the control group whereas 3-nitrotyrosine was higher in the Pb group. The authors concluded that Pb exposure increases vascular tone through nitric oxide-dependent and -independent mechanisms, increasing renal vascular sensitivity to vasoconstrictors.

4.5.3.3 Apoptosis and/or Ischemic Necrosis of Tubules and Glomeruli

Apoptosis or programmed cell death in excess can cause cell atrophy while an insufficiency can lead to uncontrolled cell proliferation, such as cancer. Pb exposure has been shown to cause morphological changes to the kidney structure. Some of these Pb-induced changes are a result of cellular apoptosis or necrosis. Past studies have shown Pb-induced necrosis in proximal tubule cells (Fowler et al., 1980). Pb-induced apoptosis is known to act through the mitochondria (Rana, 2008). Pb-induced calcium overload may depolarize the mitochondria, resulting in cytochrome *c* release, caspase activation, and apoptosis. The apoptosis is mediated by Bax translocation to the mitochondria and can be blocked by overexpression of Bcl-xl. Also, Pb-induced ALA accumulation can generate ROS, which may damage DNA leading to apoptosis.

Mitochondria are targets of Pb toxicity and often involved in apoptosis. Pb can induce uncoupling of oxidative phosphorylation, decreased substrate utilization, and modification of mitochondrial ion transport. ATP energetics are affected when ATP-Pb chelates are formed and ATPase activity is decreased. ROS formation can contribute to these mitochondrial changes and to other changes within the kidney. Antioxidant supplementation after Pb exposure can remedy some changes. All of these outcomes, in conjunction with Pb-related depletion of antioxidants (e.g., GSH) and elevation of lipid peroxidation point to possible susceptibility of the kidney to apoptosis or necrosis.

Rodriguez-Iturbe et al. (2005) reported that chronic exposure to low doses of Pb (100 ppm in drinking water for 14 weeks) results in renal infiltration of immune cells, apoptosis, NF-κB activation and overexpression of tubulointerstitial Ang(II). Similarly, higher level Pb treatment in rats (i.p. 20 mg/kg, 5 days) induced inflammatory cellular infiltrations and an increase in apoptotic cells, accompanied by more pronounced BAX

staining in kidney tubule epithelial cells (Abdel Moneim et al., 2011b). Pb treatment (0.5-1 µM) of isolated rat proximal tubular cells increased cell death by apoptosis and necrosis in a concentration- and time-dependent manner (Wang et al., 2011b). This was accompanied by increased morphological changes typical of apoptosis such as fragmented chromatin, condensed chromatin, and shrunken nuclei. These cells also exhibited decreased mitochondrial membrane potential, decreased intracellular pH, inhibition of Na^+/K^+ATPase and Ca^{2+}ATPase activity, and increased intracellular Ca^{2+} following Pb treatment.

Navarro-Moreno et al. (2009) examined the effect of 500 ppm Pb in drinking water over 7 months on the structure (including intercellular junctions), function, and biochemical properties of PT cells of Wistar rats. Pb effects in epithelial cells consisted of an early loss of the apical microvilli, followed by a decrement of the luminal space and the respective apposition and proximity of apical membranes, resulting in the formation of atypical intercellular contacts and adhesion structures. Inclusion bodies were found in nuclei, cytoplasm, and mitochondria. Lipid peroxidation (TBARS measurement) was increased in the Pb-treated animals as compared to controls. Calcium uptake was diminished and neither proline nor serine incorporation that was present in controls was noted in the PT of Pb-exposed animals. The authors speculated that Pb may compete with calcium in the establishment and maintenance of intercellular junctions.

Tubular necrosis was also observed in rats treated with Pb acetate (100 ppm, s.c.) for 30 days (El-Sokkary et al., 2005). Histological sections of kidneys from Pb-treated rats showed tubular degeneration with some necrotic cells. Similarly, El-Neweshy and El-Sayed (2011) reported glomerular damage and tubular necrosis with invading inflammatory cells after Pb treatment (20 mg/kg by intragastric feeding once daily for 60 days) to male rats. The incidence of necrosis was decreased in both of these studies by pretreatment with either melatonin or vitamin C. Pretreatment with melatonin (10 mg/kg), an efficacious free radical scavenger and indirect antioxidant, resulted in a near normal tubular structure. The authors concluded that melatonin protected the liver and kidneys from the damaging effects of exposure to Pb through inhibition of lipid peroxidation and stimulation of endogenous antioxidative defense systems (El-Sokkary et al., 2005). Vitamin C supplementation (20 mg/kg pretreatment every other day) protected the renal architecture and histology (El-Neweshy and El-Sayed, 2011).

Wang et al. (2009c) examined the effect of Pb acetate (0.25, 0.5 and 1 µM) on cell death in cultured rat primary PT cells. A progressive loss in cell viability, due to both apoptosis and necrosis, was observed in cells exposed to Pb. Apoptosis predominated and could be ameliorated with concomitant N-acetylcysteine exposure, whereas necrosis was unaffected. Elevation of ROS levels and intercellular calcium, depletion of mitochondrial

membrane potential, and intracellular glutathione levels was observed during Pb exposure. Pb-induced apoptosis was demonstrated morphologically (Hoechst 33258 staining) with condensed/fragmented chromatin and apoptotic body formation. CAT and SOD activities were significantly elevated, reflecting the response to accumulation of ROS.

4.5.3.4 Renal Gangliosides

Gangliosides are constituents of the plasma membrane that are important for control of renal GFR because they can act as receptors for various molecules and have been shown to take part in cell-cell interactions, cell adhesion, cellular recognition, and signal transduction. Aguilar et al. (2008) studied changes in renal gangliosides following Pb exposure (600 ppm Pb acetate in drinking water for 4 months) in adult male Wistar rats. Pb exposure caused an increase in blood Pb from 2.1 to 35.9 µg/dL. There was no change in serum creatinine or in hemoglobin, but there was an increase in urinary δ-ALA. The following renal gangliosides were measured by immunohistochemistry and by thin layer chromatography: GM1, GM2, GM4, and 9-O-acetylated modified form of the GD3 ganglioside (9-O-Ac-GD3). The ganglioside pattern was mainly characterized by a decrease in the GM1 ganglioside as well as by a mild increase in GM4 and GM2 gangliosides, while the strongest alteration was observed in the 9-O-Ac-GD3, which was overexpressed. The latter was observed only in the glomerular zone. This was associated with a decrease in apoptotic glomerular cells, as assessed by the TUNEL assay. The authors hypothesized that the increase in GD3-O-acetylation could represent a strategy to attenuate the normal renal apoptotic process and therefore contribute to cell survival during Pb exposure.

4.5.3.5 Altered Uric Acid

Higher occupational Pb exposure or blood Pb levels have been linked to increased risk for both gout and kidney disease (Shadick et al., 2000; Batuman, 1993). Pb is thought to alter uric acid homeostasis by affecting its kidney excretion (Emmerson and Ravenscroft, 1975; Ball and Sorensen, 1969; Emmerson, 1965). Behringer et al. (1986) suggests that the appearance of gout in patients with renal failure points to prior Pb exposure. Colleoni et al. (1993) note that in their uremic patients on regular hemodialysis blood Pb corrected for hematocrit was significantly higher than blood Pb of healthy subjects living in the same area and state that when they analyzed the possible different sources of Pb accumulation in their population they found it to be higher in patients occupationally exposed and in patients exposed to alkyl lead from atmospheric contamination from

gasoline additives. Research during the past decade indicates that uric acid is nephrotoxic at lower levels than previously recognized (Johnson et al., 2003). Therefore, the 2006 Pb AQCD (U.S. EPA, 2006b) reviewed literature implicating increased uric acid as a mechanism for Pb-related nephrotoxicity (Weaver et al., 2005a; Shadick et al., 2000). However, this does not appear to be the only mechanism, since associations between blood Pb and serum creatinine have remained significant even after adjustment for uric acid (Weaver et al., 2005a).

Alterations in serum uric acid have been studied in animal models exposed to Pb. In male rats exposed to Pb in drinking water from lactation to puberty (40 days) or post-puberty (65 days), Berrahal et al. (2011) found that plasma uric acid levels were unchanged after 40 days of exposure (puberty blood Pb level of 12.7 µg/dL) but decreased after 65 days of Pb exposure (post-puberty blood Pb level of 7.5 µg/dL); plasma urea increased after 40 days of but were unchanged after 65 days of Pb exposure (Table 4-29). Serum uric acid was increased in male rats following long-term Pb exposure (2,000 ppm for 6 weeks) (Jayakumar et al., 2009). Conterato et al. (2007) followed various parameters of kidney function after single or multiple Pb injections in rats. The single dosing regimen consisted of a single i.p. injection of 25 or 50 mg/kg Pb acetate, while the multiple injections involved once daily i.p. injection of either vehicle or Pb acetate (5 or 25 mg/kg) for 30 days. Single and multiple injections at both dose levels increased plasma uric acid levels. Similarly, Abdel Moneim et al. (2011b) reported increased serum uric acid and urea levels after 5 days of Pb acetate treatment (i.p. 20 mg/kg).

4.5.3.6 Role of Metallothionein

The metal-binding protein, metallothionein, may play a role in inclusion body formation and thus block potential interaction of Pb with cellular targets. Yu et al. (2009) described dichotomous effects of Pb acetate on the expression of MT in the liver and kidney of mice. Male mice were i.p. injected with Pb acetate in doses of 100, 200, and 300 µmol/kg and sacrificed 4, 8, and 24 hours after Pb treatment. Administration of Pb increased the levels of MT-1 mRNA in the liver and kidneys but increased MT protein only in the liver. Treatment of mouse PT cells in vitro with Pb also resulted in an increase in MT mRNA but little increase in MT protein. Thus, Pb appears to exert a dual effect on MT expression in the kidney: enhancement of MT gene transcription but suppression of MT mRNA translation. Wang et al. (2009b) did not find a change in the distribution and expression of MT-1 and MT-2 genes in the kidney of rats following long-term Pb exposure (300 ppm in drinking water, 8 weeks, serum Pb level 10.9 µg/dL), however did report a synergistic effect of Pb and cadmium on metallothionein in the kidney (see Section 4.5.4.1).

Zuo et al. (2009) examined the potential role of α-Synuclein (Scna) and MT in Pb-induced inclusion body formation. Unlike the parental wild type (WT) cells, MT-I/II double knockout (MT-null) cells did not form inclusion bodies after Pb treatment; however, transfection of MT-1 into MT-null cells allowed inclusion body formation after Pb treatment. As inclusion bodies formed during Pb treatment, soluble MT protein in WT cells was lost. As Scna is a protein with a natural tendency to aggregate into oligomers, Scna was measured in WT cells and MT-null cells after Pb treatment. In both cell lines Pb-induced Scna expression rapidly increased and then decreased over 48 hours as Pb-induced inclusion bodies were formed. Pb exposure caused increased colocalization of MT and Scna proteins and MT was localized to the surface of inclusion bodies in WT renal cortex samples following Pb treatment. Thus, Scna may be a component of Pb-induced inclusion bodies and, with MT, may play a role in inclusion body formation.

4.5.3.7 Summary

At blood Pb levels greater than 30 µg/dL, there is clear evidence in animal toxicological studies that Pb causes changes to the kidney morphology and function. Evidence for functional changes in animals following lower Pb exposure levels resulting in blood Pb levels < 20 µg/dL is generally not available. However, human epidemiologic studies have reported associations of increased blood Pb with elevated risk for kidney dysfunction in human populations with a wider range of blood Pb levels. Animal toxicological and epidemiologic studies provide a plausible biological mechanism for renal effects, including increased BP and hypertension, increased oxidative stress and inflammation, morphologic alterations, and increased uric acid.

As discussed in Section 4.5.1, the renal and cardiovascular systems are intimately linked and chronic kidney disease can be a cause, as well as a consequence of hypertension. The medical literature clearly demonstrates that renal function is inversely correlated with BP, such that an increase in BP may be accompanied by a decrease in creatinine clearance or increase in serum creatinine (Lindeman et al., 1987). Pb exposure has been causally linked to increased BP, hypertension, and other cardiovascular effects (Section 4.4). Animal toxicology studies and epidemiologic studies consistently observe associations between increases in BP and long-term Pb exposure, at relatively low (i.e., 2-10 µg/dL) concurrent blood Pb levels.

Animal studies provide evidence for concomitant vascular disease (e.g., elevated BP) and glomerular pathology following Pb exposure (Roncal et al., 2007; Bagchi and Preuss, 2005) providing a clear link between Pb exposure, cardiovascular disease, and renal dysfunction. There are various mechanisms that explain the pathway from hypertension

to kidney damage, including barotrauma to the glomerular capillaries caused by increased capillary pressure, altered vascular reactivity and vasoconstriction, altered RAAS, oxidative stress, and chronic tissue hypoxia (Palm and Nordquist, 2011). Studies in animals provide evidence for increased renal vascular reactivity (Vargas-Robles et al., 2007) and activation of the RAAS following Pb exposure (Bravo et al., 2007; Bagchi and Preuss, 2005; Sharifi et al., 2004; Carmignani et al., 1999).

Damage to the kidneys has been closely associated with increased oxidative stress and inflammation. ROS in the kidneys are responsible for preservation of routine cellular physiology and are important for cell fate, regulation of blood flow, and regulation of gene expression (Nistala et al., 2008). However, loss of redox homeostasis and excessive ROS contribute to proinflammatory and profibrotic pathways in the kidney. A number of studies have characterized the role for ROS in the pathogenesis of experimental Pb-induced renal dysfunction. For example, studies of long-term Pb exposure in animals reported elevated TBARS at a blood Pb level of 23 µg/dL (Massó-González and Antonio-García, 2009) and elevated MDA levels at a blood Pb levels of 12.7 µg/dL (Berrahal et al., 2011) (study details in Table 4-28). The increased MDA was accompanied by nephrotoxicity including decreased plasma uric acid levels, increased blood creatinine, and increased BUN. Studies have also found that treatment with antioxidant compounds may protect against Pb-induced nephrotoxicity. ROS serve as essential mediators of inflammatory signaling and thus oxidative stress is often accompanied by increased inflammation. A few studies have reported increased inflammatory markers following Pb exposure in animals, including leukocyte infiltration and increased expression of NF-κB and MCP-1 (Bravo et al., 2007; Roncal et al., 2007; Rodriguez-Iturbe et al., 2005).

Multiple ultrastructural and histological changes have been observed in the kidney following Pb exposure, including changes to the PT epithelium, endoplasmic reticulum dilation, nuclear membrane blebbing, autophagosome enlargement, and intranuclear inclusion body formation. Some of these morphological changes may be the result of cellular necrosis or apoptosis, often due to mitochondrial dysfunction, which has been observed following Pb exposure (Navarro-Moreno et al., 2009; Rodriguez-Iturbe et al., 2005; Fowler et al., 1980).

Increased uric acid has also been proposed as a mechanism for Pb-related kidney damage and may act through endothelial dysfunction, activation of RAAS, oxidative stress, and proinflammatory and proliferative events (Filiopoulos et al., 2012). Both increased and decreased uric acid has been observed in animals after exposure to Pb (Berrahal et al., 2011; Roncal et al., 2007). Epidemiologic studies provide some evidence for a positive association between Pb and increased uric acid in certain populations (Weaver et al., 2005a; Shadick et al., 2000).

In summary, various mechanisms have been characterized to explain how exposure to Pb may result in damage to the kidneys, including increased BP and hypertension, increased oxidative stress and inflammation, morphologic alterations, and increased uric acid. Studies have not investigated the dose-response relationship of all of these mechanisms; however, the various mechanisms proposed appear to have the potential to occur at all levels of exposure. This evidence supports the biological plausibility of associations observed in epidemiologic studies between Pb levels and reduced kidney function.

4.5.4 Effects of Exposure to Pb Mixtures

The effect of Pb on other cations, specifically calcium, is well established in the kidney literature. Calcium-mediated processes involving receptors, transport proteins, and second messenger signaling among other endpoints have been shown to be significantly affected by Pb exposure. The disposition of Pb in the soft tissues (kidney and spleen) can change with exposure to Pb and other compounds. Pb plus Cd exposure changed Pb disposition with increased blood Pb (versus Pb alone group) and decreased metal concentration in the kidney and liver (versus Pb alone). An Fe deficient diet significantly increased Pb deposition in adult animals (Hashmi et al., 1989), pregnant dams, and maternally-exposed fetuses (Singh et al., 1991). Dietary thiamine plus Zn slightly reduced blood and kidney Pb in exposed animals (Flora et al., 1989). Selenium (Se), a cofactor for GPx, attenuated Pb-induced lipid peroxidation and abrogated the Pb-induced attenuation of GR and SOD. Concomitant exposure to the cations aluminum and Pb protected animals from ensuing nephropathy (Shakoor et al., 2000). In summary, Pb has been shown to affect processes mediated by endogenous divalent cations. In addition, exposure to other metals or divalent cations can modulate Pb disposition and its effects in the body.

4.5.4.1 Lead (Pb) and Cadmium (Cd)

Cd shares many similarities with Pb; it has been shown to be a ubiquitous PT nephrotoxicant and accumulates in the body. Despite the similarities, few studies have evaluated associations between Cd exposure and CKD or the impact of joint exposure of Pb and Cd or other metals on CKD. As discussed in the 2006 Pb AQCD (U.S. EPA, 2006b), environmental exposure to Cd, at levels common in the U.S. and other developed countries, has been shown to impact substantially associations between indicators of Pb exposure and the kidney EBE marker, NAG, even in the presence of occupational level Pb exposure. In an occupational study, mean NAG, although higher in the Pb-exposed worker group compared to controls, was correlated with urine Cd but not blood or tibia

Pb (Roels et al., 1994). In another occupational population where both metals were significantly associated with NAG, a 0.5 μg/g creatinine increase in Cd had the same effect on NAG as did a 66.9 μg/g bone mineral increase in tibia Pb (Weaver et al., 2003a).

The 2006 Pb AQCD noted that data examining the concentration-response relationship between environmental Cd and the kidney were too scarce to determine the impact of Cd exposure on the relationship between Pb exposure and other kidney outcomes. A recent publication using NHANES data collected from 1999 through 2006 addresses this gap; (results pertaining solely to Pb were discussed in Section 4.5.2.1) (Navas-Acien et al., 2009). Geometric mean concurrent blood Cd level was 0.41 μg/L in 14,778 adults aged ≥ 20 years. After adjustment for survey year, sociodemographic factors, CKD risk factors, and blood Pb, the ORs for albuminuria (≥ 30 mg/g creatinine), reduced eGFR (<60 mL/min/1.73 m^2), and both albuminuria and reduced eGFR were 1.92 (95% CI: 1.53, 2.43), 1.32 (95% CI: 1.04, 1.68), and 2.91 (95% CI: 1.76, 4.81), respectively, comparing the highest with the lowest blood Cd quartiles. Both Pb and Cd remained significantly associated after adjustment for the other. Effect modification was not observed; however, ORs were higher for adults in the highest quartiles of both metals, compared with the ORs for the highest quartiles of concurrent blood Cd or Pb alone Table 4-25). Compared with adults with blood Cd levels ≤ 0.2 μg/L and blood Pb levels ≤ 1.1 μg/dL, adults with blood Cd levels >0.6 μg/L and blood Pb levels >2.4 μg/dL had ORs (95% CIs) of 2.34 (95% CI: 1.72, 3.18) for albuminuria, 1.98 (95% CI: 1.27, 3.10) for reduced eGFR, and 4.10 (95% CI: 1.58, 10.65) for albuminuria and reduced eGFR together. These findings are consistent with other recent publications (Akesson et al., 2005; Hellstrom et al., 2001), support consideration of both metals as independent CKD risk factors in the general population, and provide novel evidence of increased risk in those with higher environmental exposure to both metals.

However, a recent study suggests that interpretation of Cd associations with GFR measures may be much more complex. Conducted in Pb workers to address the fact that few studies have examined the impact of environmental Cd exposure in workers who are occupationally exposed to other nephrotoxicants such as Pb, the study assessed Cd dose with urine Cd, which is widely considered the optimal dose metric of cumulative Cd exposure. In 712 Pb workers, mean (SD) blood and tibia Pb, urine Cd, and eGFR using the MDRD equation were 23.1 (14.1) μg/dL, 26.6 (28.9) μg/g, 1.15 (0.66) μg/g creatinine, and 97.4 (19.2) mL/min/1.73m^2, respectively (Weaver et al., 2011). After adjustment for age, sex, BMI, urine creatinine, smoking, alcohol use, education, annual income, diastolic BP, current or former Pb worker job status, new or returning study participant, and blood and tibia Pb, higher urine Cd was associated with higher calculated creatinine clearance, eGFR (β = 8.7 mL/min/1.73 m^2 [95% CI: 5.4, 12.1] per unit

increase in logarithmic (ln)-transformed urine Cd) and logarithmic (ln)-transformed NAG, but lower serum creatinine. These unexpected paradoxical associations have been reported in a few other publications (de Burbure et al., 2006; Hotz et al., 1999) and have been observed in other populations. Potential explanations for these paradoxical results included a normal physiologic response in which urine Cd levels reflect renal filtration; the impact of adjustment for urine dilution with creatinine in models of kidney outcomes; and Cd-related hyperfiltration.

Animal studies have also examined the effects from combined exposure to Pb and Cd. Wang et al. (2009b) reported a greater than additive effect of Pb and Cd on metallothionein expression in the kidney. Long-term Cd exposure (50 ppm in drinking water, 8 weeks, serum Cd level 1.4 µg/dL) increased the expression of MT-1 and MT-2 genes in the kidney of rats, whereas Pb exposure alone did not (300 ppm in drinking water, 8 weeks, serum Pb level 10.9 µg/dL). Combined exposure to Pb and Cd increased metallothionein expression in the kidney above the level of either pollutant alone. Wang et al. (2010d) studied the effects of Pb and/or Cd on oxidative damage to rat kidney cortex mitochondria. In this study young female Sprague Dawley rats were fed for 8 weeks with either Pb acetate (300 ppm), Cd chloride (50 ppm), or Pb and Cd together in the same dosage. Lipid peroxidation was assessed as MDA content. Renal cortex pieces were also processed for ultrastructural analysis and for quantitative rtPCR to identify the mitochondrial damage and to quantify the relative expression levels of cytochrome oxidase subunits (COX-I/II/III). Cytochrome oxidase is the marker enzyme of mitochondrial function, and COX-I, II, and III are the three largest mitochondrially-encoded subunits which constitute the catalytic functional core of the COX holoenzyme. Mitochondria were altered by either Pb or Cd administration, but more strikingly by Pb plus Cd administration, as indicated by disruption and loss of mitochondrion cristae. Kidney cortex MDA levels were increased significantly by either Pb or Cd, given individually, but more so by Pb plus Cd. COX-I/II/III were all reduced by either Pb or Cd administration, but more prominently by Pb plus Cd administration. This study adds to knowledge of the combined effects of Pb and Cd on kidney mitochondria.

4.5.4.2 Lead (Pb), Cadmium (Cd), and Arsenic (As)

Wang and Fowler (2008) present a general review of the roles of biomarkers in evaluating interactions among mixtures of Pb, Cd, and As. Past studies have found that addition of Cd to treatment of rats with Pb or Pb and As significantly reduced the histological signs of renal toxicity from each element alone, including swelling of the proximal tubule cells and intranuclear inclusion body formation. On the other hand, animals exposed to Cd in addition to Pb or Pb and As showed an additive increase in the

urinary excretion of porphyrins, indicating that, although measured tissue burdens of Pb were reduced, the biologically available fraction of Pb is actually increased (Mahaffey et al., 1981; Mahaffey and Fowler, 1977).

Stress proteins were examined after exposure to mixtures of Pb and other metals. Induction of MT was strongest in groups with Cd treatment. However, co-exposure to Pb and As induced higher levels of MT protein than did either Pb or As exposure alone in kidney tubule cells. Heat shock proteins (Hsps) are commonly altered with exposure to metal mixtures. A study found in vitro (low dose) and in vivo that Pb induced Hsps in a metal/metalloid-, concentration- and time-specific manner (Wang et al., 2005a). Additive or more than additive interactions occurred among Pb, Cd, and As under combined exposure conditions.

4.5.4.3 Lead (Pb) and Zinc (Zn)

Zinc has been investigated as a protective compound against the effects of Pb. Pb treatment (35 mg/kg i.p. for 3 days) caused a significant fall in hemoglobin content, significant increases in lipid peroxidation and decreased level of reduced glutathione in liver, together with diminished total protein content in liver and kidney. Co-treatment of Pb with Zn (10 mg/kg i.p.) or ascorbic acid (10, 20 and 30 mg/kg i.p.) showed a moderate therapeutic effect when administered individually, but more pronounced protective effects after combined therapy (Upadhyay et al., 2009).

Jamieson et al. (2008) studied the effect of dietary Zn content on renal Pb deposition. Weanling Sprague Dawley rats were assigned to marginal zinc (MZ, 8 mg Zn/kg diet), zinc adequate control (CT, 30 mg Zn/kg), zinc-adequate diet-restricted (30 mg Zn/kg), or supplemental zinc (SZn, 300 mg Zn/kg) groups, with or without Pb acetate (200 ppm for 3 weeks). Pb exposure did not result in nephromegaly or histological alterations. The MZ rats had higher renal Pb (35%) and lower renal Zn (16%) concentrations than did CT rats. On the other hand, SZn was more protective than the CT diet was against renal Pb accumulation (33% lower). Standard procedures for indirect immunoperoxidase staining were used to determine MT localization in the kidney. Pb had no effect on MT staining intensity, distribution, or relative protein amounts. Western blot analysis confirmed that MT levels were responsive to dietary Zn but not to Pb exposure.

4.5.4.4 Lead (Pb) and Mercury (Hg)

Stacchiotti et al. (2009) studied stress proteins and oxidative damage in a renal-derived cell line exposed to inorganic Hg and Pb. The time course of the expression of several

Hsps, glucose-regulating proteins, and MTs in a rat proximal tubular cell line (NRK-52E) exposed to subcytotoxic doses of inorganic mercury ($HgCl_2$, 1-40 μM) and Pb ($PbCl_2$, 2-500 μM) were analyzed. ROS and reactive nitrogen species (RNS) were detected by flow cytometric analysis. Endogenous total GSH content and the enzymatic activity of GST were determined in cell homogenates. Western blot analysis and immunohistochemistry were used for quantification of Hsps and MTs. Reverse transcription PCR was used for quantification of metallothionein. The higher doses of Hg (20 μM and 40 μM) were shown to markedly inhibit growth of the cell line while the higher doses of Pb (60 μM to 500 μM) inhibited cell growth to a lesser degree. After 24 hours of exposure of 20 μM Hg, the cells presented abnormal size and pyknotic nuclei, swollen mitochondria and both apoptosis and overt necrosis. In the presence of 60 or 300 μM Pb, the cells lost cell-cell and cell-matrix contacts, showed a round size, irregular nuclear contour and often mitotic arrest, but no apoptosis or overt necrosis at 24 hours. Mercury (Hg) induced a significant increase in both ROS and RNS, maximal RNS at 24 hours, and maximal ROS at 48 hours. Pb (60 or 300 μM) did not cause an increase in ROS or RNS beyond the levels measured in control cells. Total GSH significantly increased in cells grown in the presence of Pb; the effect was concentration-dependent and GSH reached its maximal value at a dose of 300 μM Pb. The effect of Hg was biphasic: 10 μM significantly enhanced GSH by 600%, while the amount of GSH detected after 20 μM Hg only increased by 50% compared to control levels. GST activity was enhanced by both Pb and Hg. Hsp25 and Hsp72 were up-regulated by Hg but there was no effect on Grp78 as compared to control. On the contrary, Pb treatment only upregulated Grp78. Mercury (Hg) induced a time-dependent effect on MT mRNA expression, which reached its maximal value 3 hours after beginning treatment and reverted to control values at 24 hours. With Pb, on the other hand, mRNA transcription was concentration- and time-dependent. The transcripts remained overexpressed compared to controls up to 72 hours. The results of this study with regard to the Pb effect on MT synthesis clearly differ from those of Jamieson et al. (2008), which found no increase in MT following Pb exposure. This discrepancy remains to be clarified.

4.5.5 Summary and Causal Determination

Evidence from epidemiologic studies of adults, including longitudinal analyses, and a number of toxicological studies examining various exposure scenarios and lifestages suggest Pb exposure leads to reduced kidney function. However, there is uncertainty regarding the potential for reverse causality to explain findings in humans. As noted in Section 1.1 most animal toxicology studies considered relevant to this assessment report blood Pb levels up to 30 µg/dL and studies describing the mode of action are sometimes higher. The causal determination for reduced kidney function in adults is informed by evidence in humans for reduced GFR, reduced creatinine clearance, and increased serum creatinine. Biological plausibility and mode of action for these effects is provided by evidence for hypertension, oxidative stress, inflammation, vascular reactivity and injury, increased uric acid, morphological changes, and apoptosis or necrosis. The section that follows describes the evaluation of evidence for reduced kidney function, with respect to causal relationships with Pb exposure using the framework described in Table II of the Preamble. The key evidence as it relates to the causal framework is summarized in Table 4-30.

4.5.5.1 Evidence for Reduced Kidney Function

The 2006 Pb AQCD (U.S. EPA, 2006b) concluded that "in the general population, both circulating and cumulative Pb was found to be associated with a longitudinal decline in renal function," evidenced by increased serum creatinine and decreased creatinine clearance or eGFR over the follow-up of 4 to 15 years in association with higher baseline blood and bone Pb levels (U.S. EPA, 2006b). Data in general and patient populations of adults provided evidence of Pb-associated lower renal function in populations with mean concurrent or baseline blood Pb levels of 2-10 µg/dL (Akesson et al., 2005; Tsaih et al., 2004; Yu et al., 2004; Kim et al., 1996); associations with lower eGFR were observed in adults with hypertension with a mean concurrent blood Pb level of 4.2 µg/dL (Muntner et al., 2003). Since these effects on the kidney were often observed in adults with likely higher past Pb exposures, uncertainty exists as to the Pb exposure level, timing, frequency, and duration contributing to the associations observed with blood or bone Pb levels. The conclusion from the 2006 Pb AQCD was substantiated by the coherence of effects observed across epidemiologic and toxicological studies. However, a number of the animal toxicological studies were conducted at Pb exposure concentrations that resulted in blood Pb levels higher than what is relevant to the general U.S. adult human population. Both human and animal studies observed Pb-associated hyperfiltration. In rodents during the first 3 months after Pb exposure, effects were characterized by increased GFR and increased kidney weight due to glomerular hypertrophy. However,

exposure for 6 or 12 months resulted in decreased GFR, interstitial fibrosis, and kidney dysfunction. Additionally, toxicological studies found that early effects of Pb on tubular cells were generally reversible, but continued exposure resulted in chronic irreversible damage. Toxicological studies provided mechanistic evidence to support the biological plausibility of Pb-induced renal effects, including oxidative stress leading to NO inactivation. Despite the strong body of evidence presented in the 2006 Pb AQCD, uncertainty remained on the contribution of past higher Pb exposures to associations observed in adults, the impact in children, the implication of hyperfiltration, and reverse causality.

Prospective epidemiologic studies in adult men in the general population (Tsaih et al., 2004; Kim et al., 1996) support the relationship between long-term Pb exposure and reduced kidney function at exposure levels considered relevant to this assessment (≤ 30 µg/dL; see Section 1.1). These prospective population studies are high quality community epidemiologic studies that show a longitudinal association after adjustment for key potential confounding factors. As presented in Table 4-25 the slopes and 95% CI limits per 1 µg/dL increase in blood Pb level are similar in magnitude for the entire population assessed by Tsaih et al. (2004) (β = 0.0210 [-0.0019, 0.048]) and for the group of individuals with a peak blood Pb level ≤ 10 µg/dL assessed by Kim et al. (1996) (β = 0.021 [-0.005, 0.048]). In addition, these studies show an overall pattern of elevated risks across all subgroups analyzed within the population.

Cross-sectional studies of the general adult population add support to the associations observed in prospective epidemiologic studies (Section 4.5.2.1). The majority of cross-sectional studies report associations between higher measures of Pb exposure and worse renal function. These studies include analyses conducted from 1988-1994 (Muntner et al., 2003), 1999-2002 (Muntner et al., 2005) and from 1999-2006 (Navas-Acien et al., 2009) of the NHANES cohort which provides a representative U.S. population sample that may be generalizable to the total U.S. population. Re-examination of a study from the 2006 Pb AQCD (U.S. EPA, 2006b) provided data to conclude that in a population with likely higher past exposures to Pb, a 10-fold increase in concurrent blood Pb was associated with an 18 mL/min decrease in estimated creatinine clearance or a 25% decrease from the mean, and that an increase in blood Pb from the 5th to the 95th percentile (3.5 µg/dL) had the same negative impact on eGFR as did an increase of 4.7 years in age or 7 kg/m^2 in body mass index (Akesson et al., 2005). Overall, a relationship between higher Pb exposure and various indicators of lower kidney function is indicated by a set of high-quality covariate-adjusted cross-sectional studies that are conducted with different designs in different cohorts by different researchers.

A prospective study in CKD patients indicated that higher baseline blood Pb level was associated with greater CKD progression over time (i.e., reduced eGFR) (Yu et al., 2004). The use of eGFR provides a better estimate of progressive changes of renal function than creatinine clearance used in the other related studies. Additionally, body Pb burden assessed by EDTA chelatable Pb level was significantly associated with overall risk for a doubling of serum creatinine over the 4-year study period or with the need for hemodialysis.

Other studies in clinical trials of CKD patients treated with EDTA chelation provide supportive results; however, uncertainty exists due to limitations noted in the studies. Effects on renal function have also been observed in studies of CKD patient studies (Lin et al., 2006b; Lin et al., 2006a; Lin et al., 2003). It is possible, however, that the CKD patient studies, in which blood Pb level was lowered compared to control groups by chelation, demonstrate an improvement in renal function by reducing ROS, blood Pb level or both. The uncertainty related to this may reflect an involvement of both lowering of blood Pb levels and a reduction of ROS following chelation as both are possible to a varying extent. Other potential limitations in these studies include small sample size and lack of researcher blinding. The treatment protocol, which included additional calcium disodium EDTA infusions as needed based on Pb levels during follow-up, may differ from the placebo protocol; therefore, patients may have been able to discern their treatment group.

Research on the renal effects of Pb in the occupational setting has traditionally been far less consistent than that in environmentally Pb-exposed populations (Section 4.5.2.1). Limitations of the occupational evidence, including the potential for hyperfiltration, the healthy worker effect, small sample sizes, and limited control for covariates may explain this inconsistency. The actual cause of paradoxical or inverse associations (higher Pb dose with lower serum creatinine, and/or higher eGFR or calculated or measured creatinine clearance) in several of these studies is not understood. Overall, the recent occupational studies on the kidney impact of Pb exposure has been relatively consistent in reporting statistically significant associations than were data reviewed for the 2006 Pb AQCD, possibly reflecting increased reliance on EBE markers as more sensitive outcome measures, publication bias, or multiple comparisons due to a greater number of outcomes assessed.

Combined evidence from prospective and cross-sectional studies helps limit the level of uncertainty for bias from confounding with reasonable confidence. While the adjustment for specific factors varied by study, the collective body of evidence adjusted for multiple potential key confounding factors, including age, sex, BMI, comorbid cardiovascular

conditions and markers (e.g., BP, lipid levels), smoking and alcohol use, SES, medication use, and copollutant exposure.

At blood Pb levels greater than 30 µg/dL, there is clear evidence in animal toxicological studies that long-term Pb exposure causes changes to the kidney morphology and function (Khalil-Manesh et al., 1992b; Khalil-Manesh et al., 1992a). Evidence for functional changes in animals following lower Pb exposure levels resulting in blood Pb levels < 20 µg/dL is generally not available. Yet at blood Pb levels between 20 and 30 µg/dL studies with various exposure scenarios and in various lifestages provide some evidence for dysfunction in kidney function measures (e.g., decreased creatinine clearance, increased serum creatinine, increased BUN).

Animal toxicological and epidemiologic studies provide several plausible biological mechanisms for Pb-induced renal effects, including increased BP and hypertension, increased oxidative stress and inflammation, morphologic alterations, and increased uric acid (Section 4.5.3). As kidney dysfunction can increase BP and increased BP can lead to further damage to the kidneys, Pb-induced damage to either or both renal or cardiovascular systems may result in a cycle of further increased severity of disease. Pb exposure has been causally linked to both increased BP and other cardiovascular effects (Section 4.4). Notably, animal studies provide evidence for concomitant vascular disease and glomerular pathology following Pb exposure (Roncal et al., 2007; Bagchi and Preuss, 2005) providing a clear link between Pb exposure, cardiovascular disease, and renal dysfunction. Damage to the kidneys has been closely associated with increased oxidative stress and inflammation. A number of studies have characterized the role for ROS in the pathogenesis of experimental Pb-induced renal dysfunction (Berrahal et al., 2011; Massó-González and Antonio-García, 2009) and have reported increased inflammatory markers following Pb exposure in animals (Bravo et al., 2007; Roncal et al., 2007; Rodriguez-Iturbe et al., 2005). Morphological changes resulting from cellular apoptosis or necrosis and altered uric acid have also been characterized as mechanisms for Pb-induced kidney damage. As noted in Section 1.1, animal toxicology studies considered relevant to this assessment report blood Pb levels as high as 30 µg/dL and studies that describe MOA are sometimes higher. Lower concentration Pb exposures and lower blood Pb levels in animals have not been widely examined.

Several important uncertainties limit the confidence in the relationship between Pb exposure and reduced kidney function. First, because these studies report effects most often observed in adults with likely higher past Pb exposures, uncertainty exists as to the Pb exposure level, timing, frequency, and duration contributing to the associations observed with blood or bone Pb levels. Second, the potential for a bidirectional relationship because of reverse causality is plausible in observational epidemiologic

studies (see Section 4.5.2.4). While several lines of evidence support that reverse causality does not contribute substantially to associations between higher blood Pb levels and worse kidney function, reverse causation remains a plausible hypothesis. The existence of an association in adults with normal renal function does not exclude reverse causation because it is not known how Pb clearance varies within the range of normal kidney function. Thus, this bidirectional relationship is possible and additional evidence is needed to fully elucidate the extent to which diminished kidney function may itself result in increased Pb levels.

There are multiple high quality epidemiologic studies and clear biological plausibility; however, uncertainty remains regarding the potential for reverse causality to explain findings in humans. Among epidemiologic studies, key evidence is provided by the few available longitudinal studies which better characterized the temporal sequence between Pb exposure and changes in renal function by showing associations between baseline bone or blood Pb levels and reduced kidney function over time in men in the Boston, MA area and greater progression of kidney disease in CKD patients. Evidence from longitudinal studies also addressed the potential for reverse causality by showing the persistence of blood Pb-associated decrements in kidney function in the range of normal kidney function and demonstrated stronger associations than cross-sectional analyses of the same data. Cross-sectional adult studies provide supportive evidence but are weighed less than the prospective studies in conclusions because by design, they do not inform directionality (i.e., reverse causality). Inconsistencies were noted in occupational studies and studies of children, and important study design limitations were noted in clinical trials of chelation in CKD patients. These inconsistencies and limitations preclude strong inferences from the results of these three study groups. Longitudinal studies found Pb-associated decrements in renal function in populations with mean blood Pb levels of 7 and 9 µg/dL. However, the contributions of higher past Pb exposures cannot be excluded. Animal toxicological studies provide clear biological plausibility with evidence for Pb-induced kidney dysfunction at blood Pb levels greater than 30 µg/dL; however, evidence in animals with blood Pb levels < 20 µg/dL is generally not available. Still at blood Pb levels between 20 and 30 µg/dL studies provide some evidence for dysfunction in kidney function measures (e.g., decreased creatinine clearance, increased serum creatinine, increased BUN). Animal studies also provide biological plausibility for the associations observed between blood Pb levels and reduced kidney function with evidence for Pb-induced hypertension, renal oxidative stress and inflammation, morphological changes, and increased uric acid. Collectively, the evidence integrated across epidemiologic and toxicological studies, with uncertainties related to the potential for reverse causation, is suggestive of a causal relationship between Pb exposures and reduced kidney function among adults.

Table 4-31 Summary of evidence supporting renal causal determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Exposure or Biomarker Levels Associated with Effects[c]
Reduced Kidney Function – Suggestive			
Consistent associations from multiple, high quality epidemiologic studies with relevant blood Pb levels	Longitudinal epidemiologic analyses in nonoccupationally Pb exposed adults for associations with change in serum creatinine and GFR in NAS	Tsaih (2004), Kim (1996)	Median baseline blood Pb levels: Adults, 6.5 - 8.6 µg/dL[d]
	Support from prospective analyses of CKD patients and Korean workers	Yu (2004),	Mean baseline blood Pb level: 4.2 µg/dL
	Supporting cross-sectional evidence for associations between concurrent blood Pb level and serum creatinine, creatinine clearance, and GFR applying differing designs across multiple cohorts of adults in different locations.	Akesson (2005), Staessen (1992), Payton (1994)	Median concurrent blood Pb levels: Adults, 2.2 - 11.4 µg/dL
	Associations found with adjustment for potential confounding factors including age, pre-existing cardiovascular disease, baseline kidney function, and SES.	Section 4.5.2.1	
	Studies had population based recruitment (NHANES) with high follow-up participation	Muntner (2003), Navas-Acien (2009)	Mean concurrent blood Pb levels: Adults, 1.58 - 4.2 µg/dL
Important uncertainties remain	Uncertainty related to reverse causality; the bidirectional association is possible	Section 4.5.2.4	
	Uncertainty related to exposure patterns, with likely higher past Pb exposure		
	Uncertainty due to baseline serum creatinine adjustment		

Table 4-31 (Continued): Summary of evidence supporting renal causal determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Exposure or Biomarker Levels Associated with Effects[c]
Limited evidence in animals of reduced kidney function after Pb exposure	Limited evidence in animals report decreased creatinine clearance, increased serum creatinine, and decreased GFR at both relevant and high level long-term Pb exposures.	Decreased function:	Mean blood Pb levels: Rodents
		Berrahal et al. (2011)	>7.5 µg/dL (<65 days from birth)
		Roncal et al. (2007)	26 µg/dL (12 weeks as adults with pre-existing kidney disease),
		Khalil-Manesh et al.(1992a)	~30 µg/dL (6 months as adults)
		Hyperfiltration:	
		Khalil-Manesh et al. (1993a)	29 µg/dL (3 months as adults)
		No functional change:	
		Vyskocil et al. (1995)	38 µg/dL (4 months as adults)
		Ademuyiwa et al. (2009)	41 µg/dL (12 weeks as adults)
Evidence clearly describes mode of action		Section 4.5.3.7	
Hypertension	Consistent evidence for increased BP and hypertension following Pb exposure in humans and animals at relevant Pb levels across numerous studies with adjustment for potential confounding factors.	Sections 4.4 and 4.5.3.2 Table 4-24	
	Association of increased BP with reduced kidney function has been well documented	(Lindeman et al., 1987)	
Oxidative Stress	Consistent evidence for increased ROS, enhanced lipid peroxidation, and antioxidant enzyme disruption in Pb exposed animals.	Section 4.5.3.1	
Inflammation	Lymphocyte and macrophage infiltration Increased NF-κB and MCP-1 expression	Sections 4.5.2.5 and 4.2.5	

Table 4-31 (Continued): Summary of evidence supporting renal causal determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Exposure or Biomarker Levels Associated with Effects[c]
Evidence clearly describes mode of action (continued)			
Morphology	Glomerular hypertrophy Cellular apoptosis and necrosis leading to PT damage	Sections 4.5.2.5 and 4.5.3.3	
Increased Uric Acid	Evidence of increased and decreased plasma uric acid in animals. Limited evidence in humans	Section 4.5.3.5	

[a]Described in detail in Table II of the Preamble.

[b]Describes the key evidence and references contributing most heavily to causal determination and where applicable to uncertainties and inconsistencies. References to earlier sections indicate where full body of evidence is described.

[c]Describes the blood Pb levels in humans with which the evidence is substantiated and blood Pb levels in animals most relevant to this ISA.

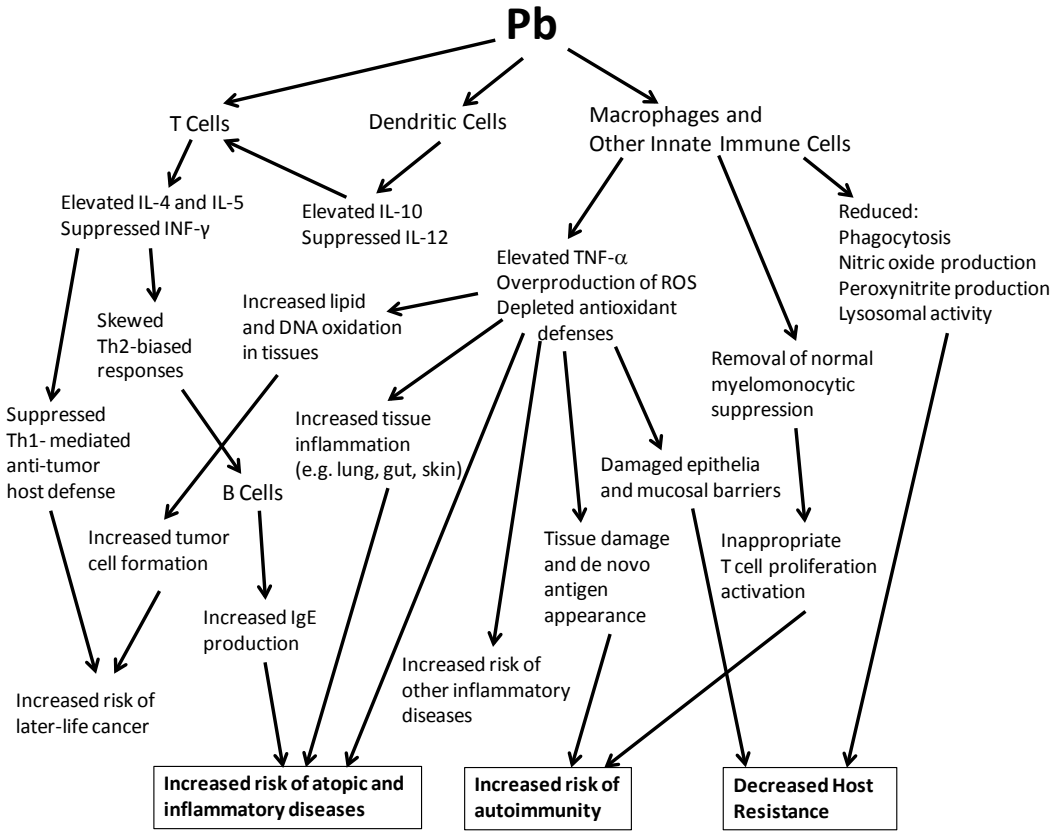
[d]Because blood Pb level in nonoccupationally-exposed adults reflects both recent and past Pb exposures, the magnitude, timing, frequency, and duration of Pb exposure contributing to the observed associations is uncertain.

4.6 Immune System Effects

4.6.1 Introduction

With respect to studies conducted in laboratory animal and in vitro models, the immune effects of Pb exposure have been extensively examined over several decades. Animal studies of the effects of Pb exposure on host resistance date back to the 1960s while those examining Pb-induced immune functional alterations, including developmental immunotoxicity, were first conducted during the 1970s. Despite this long history of research, Pb-associated immune effects in animals with blood Pb levels considered relevant to this ISA (i.e., ≤ 30 µg/dL, Section 4.1), particularly early in life, have been observed mostly within the last 10-15 years (Dietert and McCabe, 2007). Recent findings of Pb-associated changes in immunological parameters in humans have increased understanding of the immune effects of environmental exposure to Pb.

The pathways by which Pb exposure potentially may alter immune cell function and consequently increase the risk of immune-related diseases, are presented in Figure 4-33. Rather than producing overt cytotoxicity, Pb exposure has been associated with functional alterations in cellular and humoral immunity. In the 2006 Pb AQCD (U.S. EPA, 2006b), the hallmarks reported for Pb-induced changes in immune functional pathways were: (1) suppression of T cell-derived helper (Th)1-mediated immunity (i.e., suppressed Th1 cytokine production and delayed type hypersensitivity [DTH] response); (2) stimulation of Th2 immunity (i.e., increased production of Th2 cytokines and immunoglobulin (Ig)E antibody); and (3) altered macrophage function. The latter was characterized by increased production of reactive oxygen species (ROS), prostaglandin E_2 (PGE_2), and inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-6 and decreased production of nitric oxide (NO). Changes in immune cells can alter cell-to-cell interactions, multiple signaling pathways, and inflammation, that in turn, can influence the risk of developing infectious, allergic, and autoimmune diseases and exacerbate inflammatory responses in other organ systems. Studies conducted in animal and in vitro models provided consistent evidence for Pb exposure inducing effects on the range of immune effects presented in this continuum. In the much smaller epidemiologic evidence base, most studies examined Pb-exposed male workers and a limited range of immune-related endpoints.



Note: Conditions shown in boxes at the bottom are evaluated in detail in this section. As shown in the figure, immunological pathways may increase risk of diseases such as cancer (Section 4.10) and inflammatory diseases in the cardiovascular (Section 4.4), renal (Section 4.5), and hepatic (Section 4.9.1) systems. These effects are evaluated explicitly in other sections, as specified.

Figure 4-33 Immunological pathways by which Pb exposure potentially may increase risk of immune-related diseases.

Reflecting suppressed Th1 activity, toxicological evidence presented in the 2006 Pb AQCD linked Pb exposure of animals to impaired host resistance to viruses and bacteria (U.S. EPA, 2006b). Indicating a hyperinflammatory state and local tissue damage, a few available toxicological studies found Pb exposure-induced generation of auto-antibodies, suggesting an elevated risk of autoimmune reactions. Additionally, the shift toward Th2 responses suggested that Pb could elevate the risk of atopic and inflammatory responses. While the biological plausibility of such effects was supported by toxicological evidence for Pb-induced increases in Th2 cytokines, IgE, and inflammation, epidemiologic evidence was too sparse to draw conclusions about the effects of Pb exposure on these broader indicators of immune dysfunction in humans. However, in concordance with toxicological evidence, a shift to a Th2 phenotype in humans was indicated in the few available studies by associations observed between higher concurrent blood Pb level and higher serum IgE levels in children. Because of lack

of examination, the immune effects of Pb exposure in adults without occupational exposures were not well characterized.

Changes in the spectrum of immune endpoints were found in association with a wide range of blood Pb levels in animals. Juvenile and adult animals with blood Pb levels in the range of 7-100 µg/dL were found to have suppressed DTH, elevated IgE, and changes in cytokine levels. Most epidemiologic studies examined and found lower T cell abundance and higher serum IgE levels in association with population mean (or group) concurrent blood Pb levels >10 µg/dL.

With respect to important lifestages of Pb exposure, animal studies provided strong evidence for immune effects in juvenile animals induced by prenatal Pb exposures and in adult animals by postnatal exposures. There was uncertainty regarding important lifestages of Pb exposure in humans as epidemiologic studies of children primarily were cross-sectional and examined concurrent blood Pb levels. Several other limitations of epidemiologic studies were noted, including small sample sizes; little consideration for potential confounding factors such as age, sex, smoking, SES indicators, and allergen exposures; and comparisons of immune endpoints among groups with different blood Pb levels that provided little information on the concentration-response relationship.

Collectively, the small numbers of toxicological and epidemiologic studies published since the 2006 Pb AQCD, supported the previous findings of Pb-associated immune effects. Epidemiologic studies supported previous findings in children and provided new evidence for effects in nonoccupationally-exposed adults. Recent studies also expanded on the array of immunological parameters potentially affected by Pb exposure as presented in Figure 4-33. For example, a recent toxicological study indicated that Pb may modulate the function of dendritic cells. Results from recent toxicological and epidemiologic studies supported the link between Pb-associated effects on immune cells and immune- and inflammatory-related diseases by providing evidence for changes in intermediary signaling and inflammatory pathways (Figure 4-33). Several recent epidemiologic studies examined signaling molecules such as pro-inflammatory cytokines to provide coherence with toxicological findings. Recent toxicological studies further supported the broader role of Pb-associated immune modulation in mediating Pb effects in nonlymphoid tissues (e.g., nervous, reproductive, respiratory systems). Recent epidemiologic studies improved on the design of previous studies through greater examination of children and adults with blood Pb levels more comparable to current levels in the U.S. population and greater consideration for confounding by age, sex, smoking, SES indicators, and allergen exposures. This epidemiologic evidence particularly that from prospective studies, combined with the extensive toxicological

evidence formed the basis of conclusions about the immune effects of Pb exposure (Section 4.6.8).

4.6.2 Cell-Mediated Immunity

4.6.2.1 T Cells

T cells have a central role in cell-mediated immunity, influencing maturation of B cells, activation of cytotoxic T cells and macrophages, and interactions with antigen presenting cells (APCs). A majority of the evidence for the effects of Pb exposure on T cells was provided by toxicological and epidemiologic studies reviewed in the 2006 Pb AQCD (U.S. EPA, 2006b). Toxicological evidence consistently links Pb exposure with alterations in T cells with observations of Pb-induced shifts in the partitioning of CD4+ (T helper) cell populations to favor Th2 cells in vitro (25-50 µM Pb chloride, 3-5 days) (Heo et al., 1998; 1996), proliferation of Th2 cells over Th1 cells in vitro (10, 100 µM Pb chloride, 7 days) (McCabe and Lawrence, 1991), and the production of Th2 cytokines over Th1 cytokines in vitro and in vivo (wide range of Pb concentrations, Section 4.6.6.1). Epidemiologic findings are limited largely to associations observed between higher concurrent blood Pb level and lower T cell abundance in children.

In vitro results indicated various mechanisms by which Pb exposure may induce a shift to Th2 responses including activation of transcription factor NF-κB (regulates T cell activation) in cultures of human CD4+ T cells (1 µM Pb acetate, 30 minutes) (Pyatt et al., 1996) and a concentration-dependent (10, 50 µM Pb chloride, 24 hours) increased expression of MHC class II surface antigens (e.g., HLA-DR), which mediate the CD4+ response to exogenous antigens (Guo et al., 1996b). The few available recent toxicological studies described T cell-dependent and -independent pathways. Kasten-Jolly et al. (2010) provided evidence in vivo and with dietary Pb exposures relevant to this ISA. While results were based on a microarray analysis of hundreds of genes, which is subject to higher probability of chance findings, they were supported by other lines of evidence. In this study, gestational-lactational Pb acetate exposure of BALB/c mice (100 µM in drinking water of dams GD8-PND21, resultant spleen homogenate Pb level 13.8 µg/g) altered splenic cell gene expression of cytokines well documented in the literature to be affected by Pb, including the Th2 cytokine IL-4 and the Th1 cytokine interferon-gamma (IFN-γ). These changes occurred with increases in adenylate cyclase 8 and phosphatidylinositol 3-kinase in the absence of signaling molecules STAT4 or STAT6, which comprise the preferential signaling pathway for T cells. Similarly, in cultures of stimulated mouse T cells, Heo et al. (2007) showed that Pb chloride (25 µM,

12-24 hours) decreased the IFN-γ to IL-4 ratio (indicating a shift to Th2) in the absence of STAT6. Additionally, Pb blocked production of IFN-γ not by affecting gene expression but by suppressing translation of the protein. This blockage was rescued with the addition of IL-12, which is a T cell stimulating factor. The STAT results indicated a T cell-independent pathway to skewing toward Th2 responses whereas the IL-12 results pointed to a T cell-dependent pathway.

While a few available recent epidemiologic studies found associations of blood Pb levels with Th1 and Th2 cytokines in humans (Section 4.6.6.1), the extant evidence for effects on T cells in humans is derived largely from previous cross-sectional studies describing differences in the abundance of several T cell subtypes that mediate acquired immunity responses to antigens. Most studies of children found that higher blood Pb levels were associated with lower T cell abundance in serum, primarily CD3+ cells. These associations were observed in studies that adjusted for some potential confounding factors (as described below) (Karmaus et al., 2005; Sarasua et al., 2000) and studies without consideration for potential confounding (Zhao et al., 2004; Lutz et al., 1999). In children, blood Pb level was less consistently associated with abundance of other T cell subtypes such as CD4+ (helper T) or CD8+ (cytotoxic T). A few studies did not provide evidence of blood Pb-associated decreases in T cell abundance but did not consider potential confounding (Hegazy et al., 2011; Belles-Isles et al., 2002).

Associations between blood Pb level and T cell abundance were found in studies that generally had population-based subject recruitment. Most studies did not report sufficient information to assess the potential for biased participation by Pb exposure and immune conditions. Most studies had multiple comparisons; however, associations were not isolated to T cell abundance. Most studies found lower T cell abundance in groups of U.S. and non-U.S. children (ages 6 months-10 years, N = 73-331) with concurrent blood Pb levels >10 µg/dL (Zhao et al., 2004; Sarasua et al., 2000; Lutz et al., 1999). Associations were inconsistent in comparisons of children with lower blood Pb levels. Among 331 children in Germany living near (15 km) and distant from industrial facilities (some associated with organochlorine pollution), Karmaus et al. (2005) found that children (ages 7-10 years) with concurrent blood Pb levels 2.2-2.8 µg/dL (2nd quartile) had a 9 to 11% lower abundance of several T cell subtypes (for some subtypes, $p < 0.05$, t-test) compared with children with blood Pb levels <2.2 µg/dL (lowest quartile). The detection limit for blood Pb was 0.9 µg/dL. This study recruited children from schools and examined multiple exposures, reducing the likelihood of biased participation by specifically by Pb exposure. Compared with other studies of T cells, Karmaus et al. (2005) had greater consideration for potential confounding, adjusting for sex, age, number of infections in the previous 12 months, number of cigarettes/day smoked in the home in the previous 12 months, serum lipids, and blood organochlorine levels. But, SES

was not examined. Cord blood levels of organochlorine and Hg but not Pb were associated with T cell abundance in 96 newborns from a subsistence fishing community and an urban center in Quebec, Canada with a population mean cord blood Pb level <2 µg/dL (Belles-Isles et al., 2002).

Another study of children from multiple unspecified U.S. locations with and without mining and smelting operations that considered confounding also found an association between higher concurrent blood Pb level and lower T cell abundance, albeit limited to the youngest subjects (Sarasua et al., 2000). Among 241 children ages 6-35 months, a 1 µg/dL higher blood Pb level was associated with a 0.18% lower (95% CI: -0.34, -0.02) CD3+ cell count, a 0.10% lower (95% CI: -0.24, 0.04) CD4+ cell count, and a 0.04% lower (95% CI: -0.15, 0.07) CD8+ cell count, with adjustment for location of residence, age, and sex. In older age groups (36-71 months, 6-15 years), many effect estimates were positive. Analysis of blood Pb level categories indicated that associations were influenced by lower T cell abundance (3-6%) among children ages 6-35 months with concurrent blood Pb levels >15 µg/dL. Notably, 76% of subjects lived near a Pb smelting operation, were likely to have higher blood Pb levels, and may have largely influenced the observed associations. Neither Karmaus et al. (2005) nor Sarasua et al. (2000) found a monotonic decrease in T cell abundance across blood Pb level groups. Neither of these studies adjusted for SES, which has been associated with blood Pb levels and immune-related conditions such as asthma, allergy, and respiratory infections.

In the few studies with nonoccupationally-exposed adults (U.S, Italy), higher concurrent blood Pb levels were associated with higher T cell abundance (Boscolo et al., 2000; Sarasua et al., 2000; Boscolo et al., 1999), the functional relevance of which is unclear. These studies included healthy subjects and those with allergies, a wide range of samples sizes (17-433), ages (16-75 years), and mean blood Pb levels (4.3-11.4 µg/dL). Pb-exposed workers in the U.S. and Asia did not consistently have lower or higher abundance of various T cell subtypes than unexposed controls (Mishra et al., 2010; Pinkerton et al., 1998; Yücesoy et al., 1997b; Ündeger et al., 1996; Fischbein et al., 1993). The inconsistency among studies was not related to differences in sample size (20-145), age (means: 22-58 years), or blood Pb levels (14.6-132 µg/dL) among Pb workers. None of the studies of adults considered potential confounding factors, including other workplace exposures in occupational studies.

In summary, toxicological studies provided clear evidence for the effects of Pb exposure on T cells by demonstrating Pb-induced expansion of Th2 cells and increased Th2 cytokine production. Providing mechanistic evidence, a few recent toxicological studies found that Pb-induced Th2 skewing may occur via T cell-dependent (Heo et al., 2007) and -independent pathways (Kasten-Jolly et al., 2010; Heo et al., 2007). The most

consistent epidemiologic findings were associations between higher concurrent blood Pb level (>10 µg/dL) and lower T cell abundance observed in children ages 6 months to 10 years (Karmaus et al., 2005; Zhao et al., 2004; Sarasua et al., 2000; Lutz et al., 1999). An association was found with lower blood Pb levels, i.e., <3 µg/dL (Karmaus et al., 2005), albeit in children ages 7-10 years who likely had higher earlier childhood blood Pb levels. The uncertainties regarding blood Pb levels and the timing and duration of Pb exposure contributing to the associations with T cell abundance apply to the evidence as a whole since concurrent blood Pb levels in children also reflect past Pb exposures. There are several other uncertainties in this evidence, including the temporal sequence between Pb exposure and T cell changes, potential selection bias, the concentration-response relationship, and potential confounding by factors such as SES and other environmental exposures. The implications of Pb-associated changes in T cell abundance in children are limited further by the uncertain functional relevance of small magnitudes of change in T cell abundance (1-9% lower CD3+ abundance in groups of children with higher blood Pb levels) to downstream immune responses. Because toxicological studies examined effects related specifically to Th1 or Th2 responses, which have greater implications for downstream immune effects, toxicological evidence is the major consideration in drawing conclusions about the effects of Pb exposure on T cells.

4.6.2.2 Lymphocyte Activation

Lymphocytes (T, B, and natural killer [NK] cells) are activated by reversing the normal suppression mediated by macrophage-like cells. Their activation is an indicator of response to antigens. A majority of data on the effects of Pb exposure on lymphocyte activation is provided by toxicological studies reviewed in the 2006 Pb AQCD that showed both mitogen-induced expansion and suppression of alloreactive B and T lymphocytes proliferation with Pb exposures in vivo and in vitro (U.S. EPA, 2006b). Adding to the mixed nature of evidence, a recent study found that 4-week oral exposure of 7 week-old Wistar rats to 200 ppm Pb acetate induced proliferation of lymphocytes within the thymus and submaxillary lymph nodes, primarily by affecting B cells (Teijón et al., 2010). Total T cell proliferation did not change or was suppressed. Specific T cell subtypes, CD4+, CD8+ (decreased), CD4-CD8- (elevated) were affected only with i.p. Pb dosing ($p < 0.05$) but not oral exposure. Using the local lymph node assay, Carey et al. (2006) found that Pb chloride increased antigen-induced (ovalbumin, OVA) T cell proliferation in adult female BALB/c mice but administered Pb via injection (25-50 µg). The mechanistic basis for Pb effects on lymphocyte activation is not well characterized. As discussed in Section 4.6.6.2, changes in NO production appear to be involved (Farrer et al., 2008). Gao et al. (2007) described a potential role for dendritic cells. Dendritic

cells that matured in the presence of 25 µM Pb chloride enhanced alloreactive T cell proliferation in vitro compared to control dendritic cells.

A few available cross-sectional epidemiologic studies in children and nonoccupationally-exposed adults provided indirect evidence for Pb-associated lymphocyte activation. Instead of directly measuring lymphocyte proliferation, these studies measured the abundance of cells that expressed HLA-DR, a cell surface marker that indicates both activated lymphocytes and monocytes. These studies had little consideration for potential confounding, which also limits the implications of findings. In the study of children (ages 9 months-6 years, Missouri), the mean percentage of HLA-DR+ cells was about 2-fold higher ($p > 0.05$, Kruskal-Wallis) in the 19 children with concurrent blood Pb levels 15-19 µg/dL than in children with blood Pb levels 10-14 µg/dL (n = 61) or <10 µg/dL (n = 178) with adjustment for age (Lutz et al., 1999). However, activated cells were not elevated in 16 children with blood Pb levels 20-44 µg/dL. Small studies of adults without occupational Pb exposure in Italy found that concurrent blood Pb level was correlated positively with the percentage of HLA-DR expressing cells in men with and without allergies (Spearman $r = 0.51$, $p < 0.002$, n=17 each, overall median blood Pb level: 11 µg/dL, ages 19-52 years) (Boscolo et al., 1999) but only in women without allergies (Spearman $r = 0.44$, $p < 0.05$, n=25, median blood Pb level: 5.5 µg/dL, ages 19-49 years) (Boscolo et al., 2000). Associations also were found with other metals.

Comparisons of Pb-exposed workers and unexposed controls indicated similar levels of lymphocyte proliferation (≤ 1% difference) (n = 10-33, mean age: 32-40 years, blood Pb level range: 12-80 µg/dL) (Queiroz et al., 1994b; Cohen et al., 1989) or lower lymphocyte proliferation (8-25%) among Pb-exposed workers (n = 15-39, mean age: 30-49 years, mean blood Pb level: 14.6-129 µg/dL) (Mishra et al., 2003; Fischbein et al., 1993; Alomran and Shleamoon, 1988; Kimber et al., 1986). In the combined epidemiologic and toxicological evidence, Pb was associated with both activation and suppression of lymphocyte activation. The epidemiologic studies had little consideration for potential confounding factors, including other workplace exposures in occupational studies, and inconsistency among studies could not be explained by differences in sample size, age of subjects, or blood Pb level either. None of the studies provided concentration-response information. Toxicological studies have demonstrated the selective expansion of Th2 cells and suppression of Th1 cells (Section 4.6.2.1). Therefore, the differential activation of specific subtypes may not be discernible in studies that measure overall lymphocyte proliferation.

4.6.2.3 Delayed-type Hypersensitivity

Although not widely examined recently, several toxicological studies reviewed in the 2006 Pb AQCD (U.S. EPA, 2006b) and recent reviews (Mishra, 2009; Dietert and McCabe, 2007) identified a suppressed DTH response as one of the most consistently observed immune effects of Pb exposure in animal models. A recent study indicated that this effect may be mediated by dendritic cells. The DTH assay commonly is used to assess the T cell-mediated response to antigens, i.e., induration and erythema resulting from T cell activation and recruitment of monocytes to the site of antigen deposition. The DTH response is largely Th1 dependent in that Th1 cytokines induce the production of T cells specifically directed against the antigen (sensitization) and recruitment of antigen-specific T cells and monocytes to the site of antigen deposition (elicitation phase).

Previous studies demonstrated suppressed DTH in animals after gestational (Chen et al., 2004; Bunn et al., 2001a; 2001b, c; Lee et al., 2001b; Chen et al., 1999; Miller et al., 1998; Faith et al., 1979) and postnatal (McCabe et al., 1999; Laschi-Loquerie et al., 1984; Müller et al., 1977) Pb acetate exposures. Such observations were made in F344 and CD rats, BALB/c and Swiss mice, and chickens. Most studies exposed animals to Pb in drinking water and found suppressed DTH in animals with blood Pb levels relevant to this ISA (means: 6.75, 25 µg/dL) (Chen et al., 2004; Bunn et al., 2001a) and higher (51 to >100 µg/dL) (Bunn et al., 2001b; Chen et al., 1999; McCabe et al., 1999). The associations of DTH with lower blood Pb levels occurred with gestational Pb exposure.

In some studies that examined Pb exposures at multiple stages of gestation, exposures later in gestation suppressed DTH in animals (Bunn et al., 2001c; Lee et al., 2001b). These latter findings may reflect the status of thymus and T cell development. A recent study contributed to the robust evidence base by indicating a role for dendritic cells in the Pb-induced suppression of the DTH response. Gao et al. (2007) exposed bone marrow-derived dendritic cells in vitro to Pb chloride (25 µM, 10 days) then the antigen OVA and injected the cells into Pb-naïve adult mice. Mice treated with Pb-exposed dendritic cells had a diminished OVA-specific DTH footpad response compared with mice treated with dendritic cells not exposed to Pb.

Evidence indicates Pb-induced suppression of DTH in animals, some with blood Pb levels relevant to this ISA (6.75, 25 µg/dL) produced by gestational Pb exposure via drinking water of dams. The mode of action is strongly supported by observations that Pb suppresses production of the Th1 cytokine IFN-γ (Section 4.6.6.1). IFN-γ is the primary cytokine that stimulates recruitment of macrophages, a key component of the DTH response. In animal studies that also examined IFN-γ, the suppressed DTH response was accompanied by a decreased production of IFN-γ (Lee et al., 2001b; Chen et al., 1999).

Observations of a concomitant decrease in IFN-γ strengthen the link between Pb-induced inhibition of Th1 functional activities and suppression of the DTH response.

4.6.2.4 Macrophages and Monocytes

As reported in the 2006 Pb AQCD, based on a large body of toxicological evidence and some supporting epidemiologic evidence, Pb-induced alteration of macrophage function was considered to be a hallmark of Pb-associated immune effects (U.S. EPA, 2006b). Macrophages, which are produced by the differentiation of blood monocytes in tissues, mediate host defense through their role in phagocytosing pathogens and stimulating other immune cells. Pb exposure was found to induce macrophages into a hyperinflammatory phenotype as indicated by enhanced production of TNF-α, IL-6, and ROS and increased metabolism of arachidonic acid into PGE_2. Observations in macrophages of Pb-induced enhanced production of ROS, suppressed production of NO, impaired growth and differentiation, and potentially altered receptor expression [e.g., toll-like receptors]) provided coherence for the effects of Pb observed on tissue damage and diminished host resistance in animals. Several of these findings are described in detail in Sections 4.6.6.2 and 4.6.6.3. Because macrophages are major resident populations in most tissues and organs and also are highly mobile in response to microbial signals and tissue alterations, their functional impairment in response to Pb exposure may serve as a link between Pb-induced immune effects and impaired host defense, tissue integrity, and organ homeostasis in numerous physiological systems.

Some rodent studies indicated reduced macrophage generation or phagocytosis with gestational or postnatal dietary Pb acetate exposure that produced blood Pb levels (upon cessation of exposure) relevant to this ISA, i.e., 8.2 µg/dL in F344 rats (Bunn et al., 2001c) and 18 µg/dL in CBA/J mice (Kowolenko et al., 1991). Similarly, Knowles and Donaldson (1997) found that Pb acetate trihydrate given PND1 to PND21 induced a decrease in macrophage phagocytosis in turkeys but with higher blood Pb levels, 42 µg/dL. In one set of experiments, CBA/J mice exposed to Pb acetate in drinking water for 2 weeks, with blood Pb levels of 18 µg/dL, had reduced macrophage generation (Kowolenko et al., 1991) in response to *Listeria monocytogenes* inoculation (via tail vein i.v. injection), but no change in macrophage phagocytosis (Kowolenko et al., 1988). Other animal studies administered Pb through routes that may not be directly relevant to exposure routes of humans. Effects such as decreased macrophage yield, viability, phagocytosis, chemotaxis, and killing ability were reported in Swiss mice following bacterial infection and Pb treatment by oral gavage (40 mg/kg Pb nitrate, 40 days) (Lodi et al., 2011) or injection (10 mg/kg, i.p., 15 days) (Bishayi and Sengupta, 2006). Lee et al. (2002) found no change in monocyte abundance in 5-6 week-old chickens treated with

200 µg Pb acetate via the air sac in ovo at embryonic day 5 or 12. Some (Bussolaro et al., 2008; Zhou et al., 1985) but not all in vitro studies (De Guise et al., 2000) also found Pb-induced (0.2-1,000 µM Pb chloride or Pb nitrate) reduced phagocytosis. In particular, Bussolaro et al. (2008) found such effects with a relatively low concentration of Pb exposure (0.2 µM Pb nitrate, 72 hours).

The effects of Pb exposure on macrophages in humans have not been widely examined. Pineda-Zavaleta et al. (2004) was unique in examining the hyperinflammatory state specifically in macrophages, and consistent with the large body of toxicological studies, found associations of higher concurrent blood Pb level with lower NO release and higher superoxide anion release from macrophages isolated from child sera (Sections 4.6.6.2 and 4.6.6.3). These results were adjusted only for age and sex. Other studies in humans examined macrophage abundance in Pb-exposed workers, and evidence overall did not clearly indicate an association with concurrent blood Pb level. Pinkerton et al. (1998) considered potential confounding and found a <1-fold lower ($p = 0.03$) abundance of monocytes among 145 U.S. Pb smelter workers with a mean blood Pb level and age of 39 µg/dL and 33 years, respectively, than among 84 unexposed controls with a mean blood Pb level and age of <2 µg/dL and 30 years, respectively. Results were adjusted for age, race, current smoking, and workshift, but other workplace exposures were not examined. Other occupational studies did not consider potential confounding factors. Conterato et al. (2013) examined male Pb-exposed painters in Brazil with blood Pb levels 1.4-14.0 µg/dL (mean: 5.4 µg/dL), lower than those in other occupational studies. Monocyte abundance in the 50 painters (mean age: 33.5 years) was similar to that in 36 controls (mean blood Pb level: 1.5 µg/dL, mean age: 33.5 years) and 23 battery workers with much higher blood Pb levels (mean: 50 µg/dL, mean age: 37.3 years). Fischbein et al. (1993) found a <2-fold lower ($p < 0.001$) abundance of HLA-DR+ cells in two groups of New York metropolitan area firearms instructors with mean blood Pb levels of 14.6 µg/dL ($n = 36$, mean age: 49 years) and 31.4 µg/dL ($n = 15$, mean age: 48 years) than among the 36 unexposed controls (mean age: 47 years). Investigators cited other observations that 70% of monocytes express HLA-DR antigen compared with 15% of lymphocytes to attribute the reduced expression of HLA-DR in Pb-exposed workers to a reduction in activated monocytes. However, lower HLA-DR+ may have indicated reduced activation of other APCs such as dendritic or T cells.

The 2006 Pb AQCD (U.S. EPA, 2006b) also described the effects of Pb on specialized macrophages in nonlymphoid tissue, including increased ROS production and impaired phagocytosis by Kupffer cells in the liver and alveolar macrophages in the lung and suggested a link between these effects and organ/tissue dysfunction, cell death, tissue pathology and tissue-specific autoimmune reactions. The limited available recent investigation provides additional evidence for effects on Kupffer cells and alveolar

macrophages as well as microglia and astrocytes in the brain, osteoclasts in the bone, and testicular macrophages.

Fan et al. (2009b) reported major changes in phenotypic expression (e.g., CD68 and ferritin light chain), organization, and functional activity of Kupffer cells connected to apoptosis in the liver of 6-7 week old Sprague-Dawley rats treated with a single 2.5×10^4 µM Pb nitrate injection. Dosing of juvenile Wistar rats with Pb injections (15 mg/kg of Pb acetate daily for 2 weeks, resulting in a mean blood Pb level of 30 µg/dL) during early postnatal maturation resulted in an increase in GFAP, indicating chronic activation of glial cells; an increase in pro-inflammatory cytokines (i.e., IL-1β, TNF-α and IL-6); and a decrease in synaptophysin (component of presynaptic vesicles), indicating neurodegeneration in brain tissue (Strużyńska et al., 2007). Bone osteoblasts have been shown to be affected by Pb exposure (Section 4.9.4), which, given the interactions between osteoblasts and osteoclasts (Chang et al., 2008a), could have implications for development of arthritis [reviewed in Zoeger et al. (2006)]. In vitro, 1 µM Pb acetate elevated TGF-β production and cartilage formation in limb bud mesenchymal cells (Zuscik et al., 2007). Kaczynska et al. (2011) reported effects on alveolar macrophages after Pb acetate treatment (i.p. 25 mg/kg, 3 days, resulting in mean blood Pb level of 2.1 µg/dL) in Wistar rats. Macrophages infiltrated airways, limiting air space available to gas exchange and contained parts of phagocytized surfactant and alveolar lining. Resident immune cells in reproductive organs have been shown to be affected by high concentration Pb exposure. In male BALB/c mice, Pace et al. (2005) found that 0.1 ppm Pb acetate exposure in drinking water PND1-PND42 (mean peak blood Pb level: 59.5 µg/dL) resulted in sterility concomitantly with a decrease in the testicular macrophage population and an increase in apoptotic testicular cells.

In summary, an extensive toxicological evidence base demonstrates that Pb exposure decreases functionality of macrophages and promotes a hyperinflammatory phenotype. Animals with dietary Pb exposure resulting in blood Pb levels (upon cessation of exposure) relevant to this ISA, 8.2 and 18 µg/dL, had reduced macrophage generation and phagocytosis (Bunn et al., 2001c; Kowolenko et al., 1991). Some in vitro studies (Bussolaro et al., 2008; Zhou et al., 1985) provided supporting evidence. Several observations link Pb exposure to impaired function and/or structure of specialized macrophages in nonlymphoid tissue, including liver Kupffer cells and alveolar macrophages. The results suggest that immune dysfunction may contribute to the effects of Pb on dysfunction in nonlymphoid tissues and provides a link between immune dysfunction and impairments in other organ systems. However, the implications of findings to effects in humans are uncertain because in several studies, animals were treated with Pb by injection and/or had high blood Pb levels (i.e., >30 µg/dL). Evidence for Pb-induced decreases in macrophage functionality provides mode of action support

for observations of Pb-induced decreased host resistance in animals (Section 4.6.5.1). The sparse epidemiologic evidence is not conclusive but was a lesser consideration than the toxicological evidence in drawing conclusions about the effects of Pb exposure on macrophages because most epidemiologic studies did not examine the functional state of macrophages. The study that did examine the functional state of macrophages, i.e., mediators of host defense or inflammation, found an association with concurrent blood Pb level in children (Pineda-Zavaleta et al., 2004). These results were consistent with toxicological evidence but were adjusted only for potential confounding by age and sex. Occupational studies examined abundance of monocytes or markers of activated macrophages and other antigen presenting cells, and evidence did not clearly indicate a difference in Pb-exposed workers. Inconsistency among studies was not related to differences in sample size, age, or blood Pb levels of Pb-exposed workers. None of the occupational studies considered potential confounding by other occupational exposures or provided concentration-response information.

4.6.2.5 Neutrophils

In the 2006 Pb AQCD, Pb exposure was not judged to have a strong effect on neutrophils, which comprise the majority of polymorphonuclear cells (PMNs) (U.S. EPA, 2006b). This conclusion was based on the limited available toxicological evidence as compared with that for effects on other immune cells. The modulation of neutrophil activity may have important consequences on the dysregulation of inflammation and ability of organisms to respond to infectious agents. Studies of cultured human PMNs (Governa et al., 1987) and occupationally-exposed adults (Queiroz et al., 1994a; Queiroz et al., 1993; Valentino et al., 1991; Bergeret et al., 1990) found Pb-associated reductions in PMN functionality, as indicated by reduced chemotactic response, phagocytic activity, respiratory oxidative burst activity, or ability to kill ingested antigen. These observations were made in groups of Pb-exposed workers (n = 10-60) with mean ages ranging 34 to 41 years and blood Pb levels ranging 14.8 to 91.4 µg/dL. These studies reported specifically on neutrophil function not a multitude of other immune parameters. Thus, the evidence could have been influenced by publication bias. In these cross-sectional studies, the temporal sequence between Pb exposure and neutrophil function cannot be determined. Other limitations across all studies include the lack of consideration for potential confounding factors, including other workplace exposures, and high blood Pb levels of Pb-exposed workers.

Instead of examining neutrophil functional activities, the few available recent studies of animals and occupationally-exposed adults examined neutrophil counts, an increase in which has been interpreted by some investigators to be a hyperinflammatory response

and a compensatory response to Pb-induced impairment in neutrophil chemotactic activity. A study in male Wistar rats found that 12 mg Pb spheres implanted in brains (compared with control glass spheres) resulted in greater neutrophil infiltration from day 7 to 28 with inflammatory-related damage that included apoptosis and indications of neurodegeneration (Nakao et al., 2010). However, the route of Pb administration has uncertain relevance to the typical routes of Pb exposure in humans.

Occupational studies produced contrasting results that were not explained by differences in the blood Pb levels of workers. DiLorenzo et al. (2006) found a Pb-associated higher absolute neutrophil count (ANC) in analyses that adjusted for some potential confounding factors and showed a concentration-dependent relationship. In an analysis combining 68 ceramic, Pb recycling, or bullet manufacturing workers and 50 control food plant workers in Italy, a 1 µg/dL higher concurrent blood Pb level was associated with a 21.8 cells/µL (95% CI: 11.2, 32.4 cells/µL) higher ANC. While these results were adjusted for age, current BMI, and current smoking status, other workplace exposures were not examined. Pb-exposed workers had a mean age 44 years and a geometric mean concurrent blood Pb level of 20.5 µg/dL. Controls had a mean age 46.8 years and mean blood Pb level of 3.5 µg/dL. Neutrophilia (n >7,500 cells/mm^3) was found in 8 workers described to have medium to high Pb exposures (exact blood Pb levels not reported) but in no controls suggesting that long-term, higher-level Pb exposures can lead to a biologically meaningful excess of circulating neutrophils. Additionally, a blood Pb concentration-dependent relationship was indicated by observations of a monotonic increase in ANC across increasing blood Pb level groups: controls, workers with blood Pb levels ≤ 30 µg/dL, and workers with blood Pb levels >30 µg/dL. Results further indicated an interaction between concurrent blood Pb level and current smoking. ANC increased across the three blood Pb groups among current smokers but not nonsmokers. In contrast, in a study that did not consider any potential confounding factors, Conterato et al. (2013) found lower neutrophil concentrations among 23 battery workers and 50 painters in Brazil with mean concurrent blood Pb levels of 50.0 and 5.4 µg/dL, respectively, than among 36 controls with a mean blood Pb level of 1.5 µg/dL. Pb-exposed workers did not consistently have higher levels of eosinophils, basophils, monocytes, or total lymphocytes either.

Support for the decreased neutrophil function found in Pb-exposed workers is provided by findings of Pb-associated decreases in IFN-γ (Section 4.6.6.1) and complement. IFN-γ gradients serve to direct neutrophil migration. The complement system is a component of the innate immune system that is involved in chemotaxis of macrophages and neutrophils and phagocytosis of antigens. The few available occupational studies found lower complement in Pb-exposed workers with mean blood Pb levels >60 µg/dL (Ündeger et al., 1996; Ewers et al., 1982), higher than those relevant to this ISA. Neither study

considered potential confounding factors, including other workplace exposures. The evidence has limited implications also because the cross-sectional nature of studies cannot establish the temporal sequence between Pb exposure and complement. Pb has been associated with increased TNF-α (Section 4.6.6.1), which has been linked to both neutrophil stimulation and apoptosis. Therefore, it is unclear whether the evidence for TNF-α describes a potential mode of action for the effects of Pb observed on neutrophils in Pb-exposed workers.

In summary, previous occupational studies provided evidence for the effects of Pb exposure on neutrophils by finding that compared with controls, Pb-exposed workers had lower neutrophil functionality (Queiroz et al., 1994a; Queiroz et al., 1993; Valentino et al., 1991; Bergeret et al., 1990) and lower complement (Ündeger et al., 1996; Ewers et al., 1982), which is a mediator of phagocyte functionality. The limited number of recent epidemiologic studies examined only neutrophil abundance and conclusively did not find Pb-exposed workers to have higher or lower neutrophil abundance. While there is evidence for Pb-associated reduced neutrophil functionality, firm conclusions are not warranted because the results are based on cross-sectional examination of male workers with relatively high blood Pb levels (range: 18.6-100 µg/dL), and they lack consideration for potential confounding factors including other occupational exposures, concentration-response information, and analogous toxicological evidence.

4.6.2.6 Dendritic Cells

Whereas as research reviewed in the 2006 Pb AQCD (U.S. EPA, 2006b) focused on examining T cells (Section 4.6.2.1), recent ex vivo and in vitro results suggest that the effects of Pb on suppressing Th1 activity and promoting Th2 activity may be a consequence of the direct action of Pb on the function of dendritic cells, a major APC. Gao et al. (2007) found that 25 µM Pb chloride exposure for 10 days stimulated dendritic cell maturation in bone marrow cultures as shown by the increased expression of MHC class II, a cell surface marker that is used to distinguish between immature and mature dendritic cells. Additionally, upon activation with LPS, Pb-matured dendritic cells produced less IL-6, TNF-α, and IL-12 (stimulates growth and differentiation of T cells) than did control cells but the same amount of IL-10 (inhibits production of Th1 cytokines). The effect of Pb on altering the cytokine expression profile of dendritic cells, in particular, the lower IL-12/IL-10 ratio and the effect on increasing the ratio of cell surface markers CD86/CD80 were indications that Pb exposure preferentially induced development of dendritic cells that signal naïve T cell populations to shift toward a Th2 phenotype. Supporting a role for dendritic cells in skewing to a Th2 phenotype, ex vivo results from the same study showed that Pb-naïve adult BALB/c mice implanted with

Pb-exposed dendritic cells had suppressed DTH (Section 4.6.2.3) and IgG2a antibody (Section 4.6.3) responses (Gao et al., 2007).

4.6.2.7 Natural Killer Cells

Based mostly on studies reviewed in the 2006 Pb AQCD, evidence does not clearly indicate that Pb exposure affects the innate immune NK cells, which mediate host defense by killing infected cells. Some epidemiologic studies adjusted for factors such as age, sex, and smoking but did not find differences in NK cell abundance or level of functional activity by blood Pb level in children or adults with or without occupational exposures (N = 145-675) (Karmaus et al., 2005; Sarasua et al., 2000; Pinkerton et al., 1998). Studies in children did not examine potential confounding by SES, and the study in Pb-exposed workers did not examine other workplace exposures. Other smaller (N = 30-108) studies that did not consider potential confounding factors found a positive correlation between concurrent blood Pb level and NK cell abundance in adults in Italy (Boscolo et al., 2000; 1999) or reported no statistically significant association in children (quantitative results not reported) (Belles-Isles et al., 2002). Pb-exposed workers in the U.S., Europe, and Asia (N = 25-141, mean ages: 26-49 years) with higher concurrent blood Pb levels (means: 6.5-128 µg/dL) had similar means of NK cell abundance or functional activity as did unexposed controls (N = 10-84, mean blood Pb levels: <2-16.7 µg/dL, mean ages: 28-47 years) (García-Lestón et al., 2011; Mishra et al., 2003; Pinkerton et al., 1998; Yücesoy et al., 1997b; Ündeger et al., 1996; Fischbein et al., 1993; Kimber et al., 1986).

The epidemiologic evidence is not sufficiently informative for drawing conclusions about the effects of Pb exposure on NK cells because of its many limitations including cross-sectional nature, limited consideration for potential confounding, and lack of concentration-response information. However, toxicological evidence equally does not clearly indicate an effect of Pb on NK cells. A decrease in NK cell activity was found in 6-8 week-old BALB/c mice but with higher Pb exposure than that relevant to this ISA (1,300 ppm Pb acetate in drinking water, 10 days, blood Pb level ~100 µg/dL) (Queiroz et al., 2011). In an in vitro study, Fortier et al. (2008) found that Pb chloride (7.5-20.7 µg/dL) did not affect NK cytotoxicity compared with the control. However, Pb chloride was not found to affect monocytes or lymphocytes either.

4.6.3 Humoral Immunity

The 2006 Pb AQCD (U.S. EPA, 2006b) described another hallmark effect of Pb on the immune system to be an enhanced humoral immune response as characterized by increased production of IgE antibodies. Several animal and epidemiologic studies (Table 4-32) demonstrated Pb-associated increases in IgE, which mediates inflammation in allergic and allergic asthma responses by binding to mast cells and releasing histamines, leukotrienes, and interleukins upon exposure to an allergen. Neither toxicological nor epidemiologic evidence (Table 4-32) consistently indicated that Pb exposure was associated with changes in other classes of Igs including IgG, IgM, and IgA, which function in complement activation and host resistance or activation of immune cells.

4.6.3.1 Immunoglobulin E Antibody and B Cells

In toxicological evidence, there was a lack of coherence between results for IgE and activation of B cells, which regulate IgE production through differentiation into antibody-producing cells. Pb chloride exposure in vitro (10 µM up to 5 days) was found to increase markers of B cell activation, including cell surface markers and levels of plaque forming cells (PFCs), which are a measure of antibody-forming cells (McCabe and Lawrence, 1990; Lawrence, 1981a). However, several studies in animals (Swiss mice and rabbits) found a wide range of Pb exposure concentrations (0.5 to 250 ppm Pb acetate or tetraethyl Pb for 3-10 weeks, postnatal via drinking water) to decrease levels of PFCs (Blakley et al., 1980; Koller and Kovacic, 1974; Koller, 1973). Among many mice strains tested, Mudzinski et al. (1986) found an increase in PFCs only in BALB/c mice with 8-week postnatal dietary Pb acetate exposure that produced high blood Pb levels, 70 µg/dL. Epidemiologic studies of children and adults [Table 4-32 with group comparisons and (Boscolo et al., 2000; Boscolo et al., 1999) with correlation analyses] did not find a consistent association between blood Pb level and the abundance of B cells. Cell abundance may not reflect activation. Inconsistencies among studies did not appear to be related to differences in age, group blood Pb levels, or the extent of consideration for potential confounding (Table 4-32).

Most animal studies found Pb-induced increases in IgE, with key evidence provided by studies that examined Pb acetate exposure through drinking water during the gestation and/or lactation period (Snyder et al., 2000; Miller et al., 1998). In particular, Snyder et al. (2000) found elevated IgE in juvenile BALB/c mice with relevant blood Pb levels, means 5-20 µg/dL measured 0-1 week after gestational and/or lactational Pb exposure. In Miller et al. (1998), elevated IgE was found in adult mice exposed gestationally to Pb via drinking water of dams that had blood Pb levels of 30-39 µg/dL. Chen et al. (2004) did

not find gestational dietary Pb acetate exposure to result in an increase in IgE in adult F344 rats who had blood Pb levels of 6.75 and 8 µg/dL, measured one week postweaning. In BALB/c and OVA-transgenic (produce OVA-specific T cells) mice, Heo et al. (1997; 1996) found concomitant Pb-induced increases in IgE and IL-4, consistent with the mode of action of IL-4 to induce class switching of B cells to IgE producing cells. However, these effects were observed with Pb administered via s.c. injection (50 µg/100 µL, 3 times per week for 3 weeks) and with higher blood Pb levels, 38 µg/dL, than those relevant to this ISA. Some of these animal studies examined multiple immune endpoints; however, chance findings due to multiple comparisons likely are not responsible for the IgE findings because the pattern of results consistently pointed to a shift from Th1 to Th2 responses (Miller et al., 1998; Heo et al., 1997; 1996).

Observations of Pb-induced increases in IgE in animals provide biological plausibility for associations observed between higher Pb biomarkers levels and higher serum IgE levels in various populations of children (Hegazy et al., 2011; Hon et al., 2010; Hon et al., 2009; Karmaus et al., 2005; Annesi-Maesano et al., 2003; Sun et al., 2003; Lutz et al., 1999) (Table 4-32). The evidence was based on cross-sectional analyses which preclude establishing the temporal sequence between Pb exposure and IgE. Also, a monotonic blood Pb concentration-dependent increase in IgE was not consistently observed. Associations between blood Pb level and IgE were found in studies that generally had population-based recruitment. Most studies did not report sufficient information to assess the potential for biased participation by Pb exposure and immune conditions. Most studies examined multiple immune endpoints; however, associations were not isolated to IgE.

Table 4-32 Comparison of serum immunoglobulin levels and B cell abundance among various blood Pb groups.

Study	Study Population and Methodological Details	Blood Pb Level (µg/dL)	IgE[a]	IgG[b]	IgM[b]	IgA[b]	B cells[c]
Children							
Karmaus et al. (2005)	331 children, ages 7-10 yr, Hesse, Germany Cross-sectional. School-based recruitment. Moderate participation rate. Some subjects live near industrial facilities. Multiple exposures examined. Results adjusted for age, sex, #cigarettes/day smoked in home in previous 12 months, # infections in the previous 12 months, serum lipid level, blood organochlorine level. No consideration for potential confounding by SES, allergens. No monotonic C-R.	<2.2	46 (1.0)	1210	150	123	418[e] (1.0)
		2.21 - 2.83	30 (0.65)	1,214	143	121	353 (0.84)
		2.84 - 3.41	59 (1.28)	1,241	153	133	389 (0.93)
		>3.41	59 (1.28)[d]	1,201	148	136	393 (0.94)
		Means Boys: 2.8 Girls: 2.5					
Sarasua et al. (2000)	382 children, ages 6-35 mo, Multiple U.S. locations Cross-sectional. No information on participation rate. Large proportion lives near Pb and other metal sources. Comparison group age- and demographically-matched. Results adjusted for age, sex, and study location. No consideration for potential confounding by SES, allergens. Inconsistent C-R.	0.6 - 4.9		609	103	50.1	19.1 (1.0)
		5 - 9.9		666[d]	108	55.0	20 (1.05)
		10 - 14.9		680[d]	105	58.2	20.4 (1.07)
		≥ 15		630	124	61.4	22.2 (1.16)
		Mean(SD): 7.0 (5.2)					
Sarasua et al. (2000)	562 children, ages 36-71 mo, Multiple U.S. locations Same methodology as above.	0.6 - 4.9		817	120	88.6	18.4 (1.0)
		5 - 9.9		813	116	90.9	17.6 (0.96)
		10 - 14.9		856	125	96.3	19.2 (1.04)
		≥ 15		835	121	94.1	18.6 (1.01)
		Mean (SD): 6.0 (4.3)					

Table 4-32 (Continued): Comparison of serum immunoglobulin levels and B cell abundance among various blood Pb groups.

Study	Study Population and Methodological Details	Blood Pb Level (µg/dL)	IgE[a]	IgG[b]	IgM[b]	IgA[b]	B cells[c]
Sarasua et al. (2000)	675 children ages 6-15 yr, Multiple U.S. locations Same methodology as above.	0.6 - 4.9		1,031	128	140	16.1 (1.0)
		5 - 9.9		1,094[d]	131	143	15.8 (0.98)
		10 - 14.9		1,048	136	140	15.3 (0.95)
		≥ 15		1,221	106	108	20.1 (1.25)
		Mean (SD): 4.0 (2.8)					
Lutz et al. (1999)	279 children, ages 9 mo-6 yr, Springfield, MO Cross-sectional. Recruitment from public assistance and Pb poisoning prevention program. No information on participation rate. Results adjusted for age. Lack of rigorous statistical methods. No consideration for potential confounding by SES, allergens. Monotonic C-R for IgE except in highest blood Pb group.	<10	51.8 (1.0)				13.4 (1.0)
		10 - 14	74.0 (1.43)				12.6 (0.94)
		15 - 19	210.7 (4.07)				16.9 (1.26)
		20 - 69	63.7 (1.23)[d]				11.1 (0.83)
		64% with blood Pb level <10					
Hegazy et al. (2011)	318 children, ages 6 mo-7 yr, Egypt Cross-sectional. Clinic-based recruitment. No information on participation rate. Lack of rigorous statistical methods. Potential confounding not considered. No monotonic C-R.	<5	13.0 (1.0)				
		5-9	12.0 (0.92)				
		10 - 14	20.8 (1.60)				
		15 - 19	14.9 (1.15)				
		20 - 44	20.4 (1.57)				
		45 - 69	10.2 (0.78)[d]				
		63% with blood Pb level < 10					
Zhao et al. (2004)	73 children, ages 3-6 yr, Zhejiang Province, China Cross-sectional. No information on recruitment method or participation rate. Lack of rigorous statistical methods. Potential confounding not considered.	<10					16.6 (1.0)
		≥ 10					16.8 (1.01)
		Mean (SD): 9.5 (5.6)					

Table 4-32 (Continued): Comparison of serum immunoglobulin levels and B cell abundance among various blood Pb groups.

Study	Study Population and Methodological Details	Blood Pb Level (µg/dL)	IgE[a]	IgG[b]	IgM[b]	IgA[b]	B cells[c]
Sun et al. (2003)	73 children, ages 3-6 yr, Zhejiang Province, China Cross-sectional. No information on recruitment method or participation rate. Lack of rigorous statistical methods. Potential confounding not considered.	<10 ≥ 10 Mean (SD): 9.5 (5.6)	32.30 41.33[f]	40.53 34.76[f]	41.53 31.74[f]		
Adults without Occupational Pb Exposures							
Sarasua et al. (2000)	433 children and adults, ages 16-75 yr, Multiple U.S. locations Same methodology as that in children.	0.6 - 4.9 5 - 9.9 10 - 14.9 ≥ 15 Mean (SD): 4.3 (3.9)		1,099 1,085 1,231 1,169	175 175 262[d] 139	252 242 283 190	13.9 (1.0) 13.0 (0.94) 12.4 (0.89) 14.8 (1.06)
Adults with Occupational Pb Exposures							
Pinkerton et al. (1998)	84 hardware factory controls, mean age 30 yr 145 male Pb smelter workers, mean age 33 yr, U.S., exact location NR Cross-sectional. Results adjusted for age, race, current smoking status, workshift. No consideration for potential confounding by other workplace exposures, SES.	Mean: <2 Mean: 39 (cumulative over 0.5-18 yr)		1,090 1,110	94.5 106.2	180 202	14.6 (1.0) 13.2 (0.90)
Fischbein et al. (1993)	36 industrial worker controls, mean age 47 yr 36 firearms instructors, mean age 49 yr 15 firearms instructors, mean age 48 yr New York metropolitan area Cross-sectional. Lack of rigorous statistical methods. Potential confounding not considered.	NR Mean: 14.6 Mean: 31.4					8.6 (1.0) 10.5 (1.22) 11.2 (1.30)[d]
Kimber et al.	21 unexposed male controls, ages 20-60 yr	Mean: 11.8		1,062	129.4	223.5	

Table 4-32 (Continued): Comparison of serum immunoglobulin levels and B cell abundance among various blood Pb groups.

Study	Study Population and Methodological Details	Blood Pb Level (µg/dL)	IgE[a]	IgG[b]	IgM[b]	IgA[b]	B cells[c]
(1986)	39 male tetraethyl Pb plant workers, ages 25-61 yr, U.K. Cross-sectional. Lack of rigorous statistical methods. Potential confounding not considered.	Mean: 38.4		1,018	104.0	242.5	
Heo et al. (2004)	606 Pb battery plant workers, ages not reported, Korea Cross-sectional. Monotonic C-R found; lack of rigorous statistical methods. Potential confounding not considered.	<10 10-29 ≥ 30 Mean: 23	112.5 (1.0) 223.3 (1.99) 535.8 (4.76)[d]				
Anetor and Adeniyi (1998)	50 male controls, ages 22-58 yr 80 male Pb-exposed workers, ages 21-66 yr, Nigeria Cross-sectional. Lack of rigorous statistical methods. Potential confounding not considered.	Mean: 30.4 Mean: 56.3		1,997 1,188[d]	215 191	188 144[d]	
Ewers et al. (1982)	53 male various occupation controls, ages 21-54 yr 72 male Pb battery/smelter workers, ages 16-58 yr, Germany Cross-sectional. Lack of rigorous statistical methods. Potential confounding not considered.	Mean: 11.6 Mean: 51.4		190[g] 173	156[g] 127	137[g] 129	
Undeger et al. (1996)	25 male university worker controls, ages 22-56 yr 25 male Pb battery plant workers, ages 22-55 yr, Turkey Cross-sectional. Lack of rigorous statistical methods. Potential confounding not considered.	Mean: 16.7 Mean: 74.8		1,202 855[d]	140 93.3[d]	210 168	636[e] (1.0) 546[e] (0.86)

Table 4-32 (Continued): Comparison of serum immunoglobulin levels and B cell abundance among various blood Pb groups.

Study	Study Population and Methodological Details	Blood Pb Level (µg/dL)	IgE[a]	IgG[b]	IgM[b]	IgA[b]	B cells[c]
Alomran and Shleamoon (1988)	18 management personnel controls	NR		1,713		183	
	39 Pb battery workers, mean age 35 yr Iraq Cross-sectional. Controls age matched. Lack of rigorous statistical methods. Potential confounding not considered.	64 (previously reported)		1,610		170	

Note: Results are grouped according to strength of study design and methodology including the extent of potential confounding considered and the relevancy of the examined blood Pb levels to current levels in the general U.S. population.

[a]IgE data are presented as IU/mL unless otherwise specified. In parentheses are ratios of IgE in the higher blood Pb group to IgE in the lowest blood Pb group.

[b]Other Ig data are presented as mg/dL unless otherwise specified.

[c]B cell data are presented as the percentage of B cells among all lymphocytes unless otherwise specified. In parentheses are the ratio of B cells in the higher blood Pb group to B cells in the lowest blood Pb group.

[d]$p < 0.05$ for group differences.

[e]Data represent the number of cells/µL serum.

[f]Data represent the mean rank for Mann-Whitney U test, $p = 0.07$ for IgE.

[g]Data are presented as IU/mL.

Karmaus et al. (2005) had greater adjustment for potential confounding factors and examined associations with lower blood Pb levels than did other studies. The detection limit for blood Pb was 0.9 µg/dL. Compared with 82 children (ages 7-10 years) in Germany with concurrent blood Pb level <2.2 µg/dL, children with blood Pb levels 2.84-3.41 µg/dL (n = 86) and >3.4 µg/dL (n = 82) had 28% higher serum IgE levels (p = 0.03, F-test). These differences were found with the adjustment for age, serum lipid levels, number of infections in the previous 12 months, number of cigarettes/day smoked in the home in the previous 12 months, and blood organochlorine levels. Some children lived near organochlorine-polluting facilities. SES was not examined. Although IgE was elevated in children with relatively low blood Pb levels (>2.84 µg/dL), in these children ages 7-10 years, the contribution of higher past Pb exposures cannot be excluded. Similar differences in IgE count on basophils were not found among the blood Pb quartiles. Although serum IgE and basophil-bound IgE have been correlated in adults (Malveaux et al., 1978; Conroy et al., 1977), few data are available in children (Dehlink et al., 2010). A study in children found that serum IgE levels were not correlated with basophil-bound IgE (Spearman r = -0.003) but were correlated with other IgE receptor-expressing cells such as dendritic cells and monocytes (Spearman r = 0.43 to 0.65, p <0.05) (Dehlink et al., 2010). The number of IgE-bound basophils also has been highly variable across individuals, particularly children (Hausmann et al., 2011; Dehlink et al., 2010). Thus, it is not unexpected that higher blood Pb level was associated with higher serum IgE but not basophil-bound IgE counts in Karmaus et al. (2005). In this study, blood Pb level was not associated with serum levels of IgG, IgA, or IgM or B cell abundance. Lutz et al. (1999) found higher serum IgE in low SES children (9 months-6 years) on public assistance in Springfield, MO after only adjusting for age, albeit with concurrent blood Pb levels >10 µg/dL (n = 105/279).

Recent studies in children (non-U.S.) also reported associations between concurrent blood Pb level and elevated serum IgE but did not adjust for potential confounding factors (Hegazy et al., 2011; Hon et al., 2010; Hon et al., 2009). Hon et al. (2010; 2009) demonstrated associations in 110 children (mean age 9.9 years) with atopic dermatitis in Hong Kong with low blood Pb levels (range: 1.4-6.0 µg/dL) and found that blood Pb level also was correlated with severity of atopic dermatitis, a condition commonly characterized by elevated IgE levels. Among 318 children, ages 6 months to 7 years, in Egypt, Hegazy et al. (2011) did not find a monotonic increase across the blood Pb groups.

While most studies of children examined concurrent blood Pb, Annesi-Maesano et al. (2003) found that Pb level in infant hair (mean: 1.38 ppm, n = 234) but not cord blood (mean: 6.7 µg/dL, n = 326) or placenta (mean: 9.6 µg/dL, n = 332) was associated with cord serum IgE in newborns in Paris. While this study better indicated the temporal sequence between Pb exposure and an increase in IgE, an important limitation was a lack

of consideration for potential confounding. The authors inferred a stronger effect of Pb exposure integrated over the entire prenatal period than exposure closer to birth. But, there is no empirical basis for interpreting hair Pb levels (Section 3.3.4.2). Cotinine was not associated significantly with hair Pb or IgE. The hair Pb-cord blood IgE correlation was larger among the 67% infants with mothers without allergies (Spearman r = 0.21, p <0.01) than infants with maternal allergies (Spearman r = 0.12), pointing to a possible masking of a Pb association by the stronger association of family history of allergy.

Blood Pb level also was associated with IgE in adults without (Pizent et al., 2008) and with occupational Pb exposure (Heo et al., 2004). Pizent et al. (2008) adjusted for potential confounding by age, pack years smoking, and alcohol consumption and found that higher concurrent blood Pb level was associated with higher IgE in 166 women in Zagreb, Croatia of similar SES (i.e., white-collar office workers) (Pizent et al., 2008). Among women not on hormone replacement therapy or oral contraceptives, a 1 µg/dL higher blood Pb level was associated with a 0.60 (95% CI: 0.58, 1.18) higher log of IgE. Concurrent blood Pb levels were low in these women who were aged 19-67 years (mean: 2.16 µg/dL, range: 0.56-7.35 µg/dL); however, the cross-sectional study design makes it difficult to characterize the temporal sequence between exposure and outcome or the timing, level, frequency, and duration of Pb exposure that contributed to the observed association. Authors did not report an effect estimate in men because it did not attain statistical significance. Without quantitative results, it is difficult to ascertain whether there was suggestion of association in men but insufficient power to indicate statistical significance due to the smaller number of men examined (50 men versus 166 females). Another study of 34 men with and without allergy in Italy also did not report quantitative results and only indicated a lack of statistically significant correlation between concurrent blood Pb level (median: 11 µg/dL) and IgE without considering potential confounding (Boscolo et al., 1999).

Limitations of the collective epidemiologic evidence for IgE include the cross-sectional analyses with limited adjustment for potential confounding. Karmaus et al. (2005) found a blood Pb-IgE association with adjustment for age, blood organochlorine levels, serum lipid levels, number of infections in the previous 12 months, and number of cigarettes a day smoked in the home in the previous 12 months. Lutz et al. (1999) comprised a low SES population on public assistance; however, none of the studies adjusted for SES or allergen exposure. Lower SES has been associated with poorer housing conditions, higher exposures to Pb, allergens, and other factors associated with allergy and asthma. Allergen exposure and lower SES are associated with higher IgE and related conditions such as allergy and asthma (Bryant-Stephens, 2009; Dowd and Aiello, 2009; Aligne et al., 2000). Most studies did not provide detailed demographic or residential information.

Thus, there is uncertainty as to the extent to which the blood Pb-IgE associations observed in children may be confounded by unmeasured SES and/or allergen exposure.

For the cross-sectional evidence for IgE, consideration is given to the possibility of associations being influenced by reverse causation, i.e., the possibility that higher IgE results in greater inflammation and consequently, greater lung permeability and greater absorption of Pb into the blood. Studies have not directly compared Pb uptake and transfer into blood in groups with different IgE levels but have assessed lung epithelial permeability by examining lung clearance of inhaled very small, hydrophilic radiolabeled solutes. Compared with controls, animals sensitized with allergens to produce higher IgE had similar clearance from lungs (Turi et al., 2011). Histamine was shown only transiently to increase lung clearance of ^{99m}Tc-DTPA particles (492 daltons, 0.57 nm) in a baboon (Yeates and Hameister, 1992) and in humans, in both those with and without asthma (Rees et al., 1985; Braude et al., 1984; O'Byrne et al., 1982). Histamine is released by IgE-bound mast cells and basophils upon exposure to sensitized antigens and leads to inflammation. Increased lung clearance of inhaled ^{99m}Tc-DTPA particles, and in some cases, increased presence in blood, has been found in some (Ilowite et al., 1989; Rees et al., 1985; O'Byrne et al., 1984) but not all (Del Donno et al., 1997; Elwood et al., 1983) studies of subjects with asthma (n = 9-13 across studies) and also with airway inflammation associated with fibrosis, smoking, and pulmonary edema (U.S. EPA, 2009a). The available evidence does not inform Pb absorption directly but provides some indication of higher inflammation resulting in increased lung permeability to very fine particles. However, a role for reverse causation may apply particularly to very fine particulate Pb and exposure via inhalation. Evidence does not indicate inhalation to be a significant route of exposure compared with ingestion (Section 3.1), and a relationship between IgE-mediated inflammation and gastrointestinal permeability has not been indicated. Thus, there is not an empirical basis for attributing the blood Pb-IgE associations observed in children or adults to reverse causation.

4.6.3.2 Other Immunoglobulin Antibodies

A small number of available recent toxicological studies examined IgG subtypes, and as in previous studies, found inconsistent effects of Pb exposure. Kasten-Jolly et al. (2010) examined 100 µM Pb acetate in drinking water of BALB/c dams GD8-PND21 because it produced relevant blood Pb levels in another study, i.e., 10-30 µg/dL (Snyder et al., 2000). Pb-exposed pups had increases in the expression of genes encoding Ig antibodies and those involved in B lymphocyte function and activation. These included genes for the heavy chain of IgM, IL-4, IL-7 and IL-7 receptor, IL-21, RAG-2, CD antigen 27, B-cell leukemia/lymphoma 6, RNA binding motif protein 24, Histocompatibility class II antigen

A (beta 1), Notch gene homolog 2, and histone deacetylase 7A. These results were produced by a microarray analysis of hundreds of genes, which is subject to a higher probability of finding an effect by chance.

Other recent animal studies examined specific IgG subtypes, did not find Pb-induced changes in a consistent direction, and thus did not clearly indicate a shift to Th2 responses. All animal studies were limited by the higher blood Pb levels of animals than those relevant to this ISA. Fernandez-Cabezudo et al. (2007) reported evidence for a shift to Th2 responses following *Salmonella* infection in C3H/HeN mice exposed postnatally to 1×10^4 µM Pb acetate in drinking water for 16 weeks (resultant mean blood Pb level: 106 µg/dL). Relative to control mice, production of the Th2 cytokine IL-4 increased in spleen cells of Pb-exposed mice after infection as did serum levels of *Salmonella*-specific IgG1. Infection increased Th1-mediated IgG2a levels in control but not Pb-exposed mice. In contrast, Gao et al. (2006) found a Pb-induced increased IgG2a/IgG1 ratio, albeit via i.p. injection (50 µg Pb chloride, 3 times per week for 3 weeks), high blood Pb levels (65 µg/dL), and in a highly-specialized strain of adult knockout mice without ability to produce IFN-γ. This result was surprising given evidence that IFN-γ usually directs secretion of IgG2a; however, the authors suggested that in these knockout mice, Pb may initiate a Th1 response via an IFN-γ independent pathway to enhance IgG2a production. Carey et al. (2006) found concentration-dependent increases in both IgG2a- and IgG1-producing cells (after 7 days) in adult BALB/c mice treated with subsensitizing doses of a T cell-independent (Trinitrophenyl-Ficoll [TNP-Ficoll]) or T cell-dependent (TNP-ovalbumin [TNP-OVA]) hapten-protein conjugate and 25-50 µg Pb chloride by injection. These results indicated stimulation of both Th1- and Th2-mediated mechanisms. Pb treatment also increased the numbers of T and B cells and IgM-producing cells in the lymph node against both TNP-Ficoll and TNP-OVA. The increase in IgM-producing cells against TNP-Ficoll indicated a T-cell independent mechanism. Despite finding increases in both IgG1- and IgG2a-producing cells, the authors concluded that Pb induced Th2 skewing based on observations of Pb-induced increases in T and B cells and suppression of DTH. Thus, results indicated the potential for Pb to promote allergic sensitization against T-dependent antigens.

Some (Belles-Isles et al., 2002; Sarasua et al., 2000) but not all (Karmaus et al., 2005), studies in children found blood Pb-associated increases in IgG but had limited implications because of the lack of extensive consideration for potential confounding factors. Sarasua et al. (2000) adjusted for age, sex, and location and found associations of higher concurrent blood Pb level with higher IgA, IgG, and IgM in 372 U.S. children ages 6-35 months but not older (36-71 months, 6-15 years, 16-75 years, n = 433-673). In the youngest age group, a 1 µg/dL higher blood Pb level was associated with a 0.8 [95% CI: 0.2, 1.4], 4.8 [95% CI: 1.2, 8.4], and 1.0 [95% CI: 0.1, 1.9] mg/dL higher IgA, IgG,

and IgM, respectively. In the youngest children, serum levels of all three examined Igs were higher among the 24 children with concurrent blood Pb levels ≥ 15 µg/dL than among the 165 children with blood Pb levels <5 µg/dL. Examining cord blood Pb, Belles-Isles et al. (2002) better indicated the temporal sequence between Pb exposure and IgG levels. Among 97 newborns in Quebec, Canada from subsistence fishing communities and two other towns (geometric mean blood Pb levels: 1.64 and 1.32 µg/dL, respectively), higher cord blood Pb level was associated with higher cord serum IgG adjusted for prenatal maternal smoking status. However, IgG also was associated with cord plasma organochlorines, which also are elevated with high fish diets.

Most examination of the effects of Pb on IgA, IgG, and IgM in humans is provided by previous studies of Pb-exposed workers (mostly males) from various industries with mean ages 32-36 years and mean blood Pb levels 38-74.8 µg/dL (Anetor and Adeniyi, 1998; Pinkerton et al., 1998; Ündeger et al., 1996; Queiroz et al., 1994b; Alomran and Shleamoon, 1988; Kimber et al., 1986; Ewers et al., 1982). As are toxicological findings for these other Ig classes, epidemiologic evidence is mixed, with higher, lower, and similar Ig levels found in Pb-exposed workers (n = 25-145) compared with unexposed controls (n = 18-84). Some studies reporting lower Ig levels in Pb-exposed workers included workers with the highest mean blood Pb levels (>50 µg/dL) (Anetor and Adeniyi, 1998; Ündeger et al., 1996; Ewers et al., 1982). The lack of analysis of potential confounding factors, including other workplace exposures, precludes characterization of others factors that may contribute to inconsistent associations in occupational studies.

4.6.3.3 Summary of Humoral Immunity

Evidence in support of the effects of Pb exposure on humoral immunity largely comprises consistent toxicological and epidemiologic observations of Pb-associated increases in IgE. The combined toxicological and epidemiologic results do not clearly indicate the effects of Pb exposure on IgG, IgM, or IgA.

Collectively, epidemiologic studies conducted in the U.S., Europe, and Asia, some with large study populations (n = 279-331) indicate higher serum IgE in children with concurrent blood Pb levels >10 µg/dL. There was some evidence of associations in non-U.S. children with lower blood Pb level; however, the contribution of higher Pb exposures earlier in childhood cannot be excluded (Hon et al., 2010; Karmaus et al., 2005). Pb-associated increases in IgE were found in children with atopic dermatitis (Hon et al., 2010; 2009) and children without immune conditions (Sun et al., 2003). Other studies did not report the health status of subjects. Thus, sufficient information was not reported to assess the potential for selection bias. Among studies that provided

concentration-response information, most did not find serum IgE to increase monotonically across blood Pb groups (Hegazy et al., 2011; Karmaus et al., 2005; Lutz et al., 1999). All evidence in humans is based on cross-sectional analyses, making it difficult to establish the temporal sequence between Pb exposure and increase in IgE. Some but not all evidence points to increased lung permeability to very small particles in the presence of acute airway inflammation or inflammatory conditions, but there is a lack of evidence for increased gastrointestinal permeability for the more dominant ingestion route of Pb exposure. Thus, reverse causation seems unlikely as the primary explanation for the observed blood Pb-IgE associations. Most studies did not consider potential confounding, and none adjusted for SES. A study in children in Germany and a study in adults in Croatia adjusted for age and smoking, with additional adjustment for blood organochlorine levels in children (Karmaus et al., 2005) and alcohol consumption in adults (Pizent et al., 2008). Blood Pb level was associated with IgE in a low SES population of children in Michigan (Lutz et al., 1999) and in a population of female office workers of similar SES in Croatia (Pizent et al., 2008). However, uncertainty remains regarding confounding in the associations observed between blood Pb level and IgE in humans. Biological plausibility for the epidemiologic evidence is provided by the Pb-induced increases in IgE observed in most of the animal studies, with some evidence at blood Pb levels relevant to this ISA (Snyder et al., 2000; Miller et al., 1998). Toxicological evidence indicates increases in IgE with gestational and/or postnatal juvenile Pb exposure, whereas epidemiologic evidence points to associations with concurrent blood Pb levels. Because concurrent blood Pb levels in children reflect both recent and past Pb exposures, the combined evidence indicates that Pb exposures during childhood may affect IgE levels. While toxicological evidence for B cell activation is inconsistent, the mode of action for Pb-induced IgE production is well supported by extensive toxicological evidence for Pb-induced increases in the Th2 cytokine, IL-4 (Section 4.6.6.1). The coherence between epidemiologic and animal findings for IgE and evidence describing modes of action for increases in IgE supports a relationship between Pb exposure and increases in IgE.

4.6.4 Inflammation

The 2006 Pb AQCD (U.S. EPA, 2006b) identified misregulated inflammation a major immune-related effect of Pb based primarily on consistent toxicological evidence for Pb-induced increases in pro-inflammatory cytokines (Section 4.6.6.1), PGE_2, and ROS (Section 4.6.6.3). Inflammation has been characterized as a major mode of action for Pb effects in multiple organ systems such as the liver, kidney, and vasculature given that immune cells make up permanent residents and infiltrating cell populations of these other

organ systems (Section 4.2.5). Inflammation also provides a link between the evidence for the effects of Pb on modulating function of immune cell and production of cytokines and IgE and the evidence for the effects of Pb on immune-based conditions such as infections and asthma and allergy. For example, IL-4-induces increases in IgE, which primes basophils and mast cells to secrete histamine, leukotrienes, and cytokines, which in turn, produce the inflammatory-related effects associated with asthma and allergy, i.e., airway responsiveness, mucus secretion, respiratory symptoms. Pb-induced inflammation also has been associated with diminished host resistance by inducing local tissue damage. As described in Section 4.6.6, the few available recent toxicological studies support the effects of Pb exposure on inflammation with findings of Pb-induced increases in pro-inflammatory cytokines, ROS and PGE_2.

The few available epidemiologic studies have found Pb-associated changes in ROS release from macrophages (Section 4.6.6.3) and cytokine levels (Section 4.6.6.1) in children and adults. Adding to this evidence, recent cross-sectional studies found associations between blood Pb level and indicators of inflammation that may be related to multisystemic effects. As discussed in Section 4.6.3.1, some but not all evidence has indicated increased lung permeability in the presence of acute inflammation or various inflammatory conditions, but evidence is lacking for a relationship with gastrointestinal permeability for the more dominant ingestion route of Pb exposure. Thus, reverse causation seems unlikely as the primary explanation for the observed blood Pb-inflammation associations. However, because of the cross-sectional design of studies, the temporal sequence between Pb exposure and inflammation cannot be established. The most compelling epidemiologic evidence was provided by studies in adults, which were larger and had greater consideration for potential confounding. The consistent pattern of association observed across the multiple comparisons made reduces the likelihood of finding associations due to chance alone.

Strengths of the 1999-2004 NHANES analysis (age $\geq$ 40 years, N = 4,663-7,342) included the examination of several potential confounding factors, multiple exposures and outcomes in predominately healthy adults and statistical analyses to provide nationally-representative results. Higher concurrent blood Pb level was associated with higher serum inflammation markers, C-reactive protein (CRP), fibrinogen, and white blood cell (WBC) count among men (Songdej et al., 2010). Results were adjusted for age; sex; race/ethnicity; education; current income; physical activity; and several factors related to inflammation including, BMI, smoking status, and history of diabetes or inflammatory or cardiovascular disease. For women, most ORs for associations between quintiles of blood Pb and tertiles of CRP, fibrinogen, and WBC count were <1.0 whereas corresponding ORs in men mostly were >1.0. For example, compared with men with concurrent blood Pb level <1.16 µg/dL (detection limit: 0.6 µg/dL), men with blood Pb

levels of 1.16-<1.63 µg/dL, 1.63-<2.17, 2.17-<3.09 µg/dL, and ≥ 3.09 µg/dL had elevated odds of higher CRP (OR [95% CI]: 2.22 [1.14, 4.32], 1.67 [0.85, 3.28], 2.12 [1.07, 4.21], and 2.85 [1.49, 5.45], respectively). Although the highest ORs were found in the highest blood Pb quintile (≥ 3.09 µg/dL), monotonic concentration-dependent increases were not observed. Consistent with NHANES findings, higher concurrent blood Pb level was associated with higher levels of WBCs and IL-6 with adjustment for age, BMI, and current smoking status among 300 university students in Incheon, Korea (Kim et al., 2007). Adults with allergic conditions or using anti-inflammatory medication were excluded; however, sufficient information was not reported to assess potential selection bias. Larger effects were estimated for the 147 men in the upper two quartiles of blood Pb levels, 2.51-10.47 µg/dL than for the full range of blood Pb levels (N = 300).

Low blood Pb levels also were associated with inflammation in a small genome-wide association study that included 37 children with autism and 15 children without autism (ages 2-5 years; blood Pb levels: 0.32-5.2 µg/dL, detection limit: 0.06 µg/dL) in California. The subjects' ages overlap with those associated with peak blood Pb levels, reducing the likelihood of subjects having much higher past blood Pb levels. In models that included age, sex, and autism diagnosis, concurrent blood Pb level was associated with the expression of several immune and inflammation genes, including HLA-DRB and MHC Class II-associated invariant chain CD74 (influences antigen presentation) (Tian et al., 2011). Blood Pb levels were similar between children with and without autism. Correlations with gene expression were observed in both groups, but they were in opposite directions (positive in children with autism and negative in children without autism). With gene expression arrays, there is a higher probability of finding associations by chance. Further, there was limited consideration for potential confounding, and the representativeness of findings in children with autism may be limited. However, the results are supported by observations that Pb chloride (10-100 µM) increases MHC expression in mouse and human HLA APCs (Guo et al., 1996a; McCabe and Lawrence, 1991).

In summary, Pb-associated increases in indicators of inflammation such as CRP, WBCs, and IL-6 were found in populations mostly comprising healthy adults with concurrent blood Pb levels 1.16-10 µg/dL with adjustment for several potential confounding factors (Songdej et al., 2010; Kim et al., 2007). The analysis of adults participating in NHANES was particularly noteworthy for its representative population and adjustment for age, sex, race/ethnicity, education, current income, physical activity, and several inflammatory conditions (Songdej et al., 2010). Because all evidence was based on cross-sectional analyses, the relative contributions of recent and past Pb exposures to the observed associations are uncertain. Other lines of evidence do not strongly support reverse causation as the primary explanation for blood Pb-inflammation associations given the

multiple routes of Pb exposure; however, the temporal sequence between Pb exposure and inflammation is difficult to establish. Despite the limited extent and cross-sectional nature of epidemiologic evidence, the biological plausibility is provided by findings of Pb-induced increases in Th2 cell partitioning (Section 4.6.2.1) and IL-6 (Section 4.6.6.1) in toxicological studies. Th2 cells produce IL-6 which is the primary stimulus for expression of CRP and fibrinogen (Hage and Szalai, 2007; Fuller and Zhang, 2001).

4.6.5 Immune-based Diseases

4.6.5.1 Decreased Host Resistance

The capability of Pb to reduce host resistance of animals to bacteria has been recognized for almost 40 years and was supported by several animal studies described in the 2006 Pb AQCD. These studies demonstrated increased mortality in animals following Pb exposure through drinking water and infection with *Listeria monocytogenes*. Multiple investigations in the same laboratory indicated increases in body burdens of viable bacteria, mortality, and sickness behavior induced by *Listeria* exposure in juvenile or adult BALB/c or CBA/J mice exposed postnatally to 500 to 2,000 µM Pb acetate in drinking water for 3 to 8 weeks (Dyatlov and Lawrence, 2002; Kim and Lawrence, 2000; Kishikawa et al., 1997; Lawrence, 1981b). Decreased bacterial resistance was observed in mice with blood Pb levels (upon cessation of Pb exposure) relevant to this ISA, i.e., 25 µg/dL in BALB/c mice exposed PND1-PND22 (Dyatlov and Lawrence, 2002) and 20 µg/dL in adult C3H/HeN mice with 16-week exposure (Fernandez-Cabezudo et al., 2007). Other studies found mortality from *Salmonella* or *E. coli* or reduced clearance of *Staphylococcus* in mice or rats administered Pb acetate or nitrate via injection, a route of Pb exposure less relevant to humans (Bishayi and Sengupta, 2006; Cook et al., 1975; Hemphill et al., 1971; Selye et al., 1966). Although not examined as much, increased mortality from viral infection was found in mice and chickens with postnatal dietary Pb (mostly Pb acetate) exposure for 4-10 weeks (Gupta et al., 2002; Youssef et al., 1996; Exon et al., 1979; Thind and Khan, 1978; Gainer, 1977). These effects were observed in animals with high blood (71-313 µg/dL) (Gupta et al., 2002; Thind and Khan, 1978) or tissue (0.12-0.71 ppm) (Exon et al., 1979) Pb levels.

The mode of action for Pb-induced decreased host resistance is well characterized by observations that Pb suppresses Th1-driven acquired immune responses and increases inflammatory responses in target tissue, which may compromise host protective barriers. Host resistance to bacteria such as *Listeria* requires effective Th1-driven responses including the production of IL-12 and IFN-γ (Lara-Tejero and Pamer, 2004) and these

have been found to be inhibited by Pb exposure (Section 4.6.6.1). The lack of IFN-γ can inhibit appropriate and timely macrophage activation. Strengthening the evidence for Pb-induced decreased host resistance, Fernandez-Cabezudo et al. (2007) found both increased mortality and decreased production of IL-12 and IFN-γ (ex vivo in spleen cells) in *Salmonella*- plus Pb-exposed CH3/HeN mice with blood Pb levels of 20 µg/dL. Nitric oxide is produced by activated macrophages and has been found to be suppressed by Pb exposure (Section 4.6.6.2). Pb-induced decreases in bacterial clearance have been found in conjunction with reduced NO and macrophage functionality (Bishayi and Sengupta, 2006). Further, Pb-induced inflammation has been demonstrated as increases in ROS and PGE_2 (Section 4.6.6.3). Additional mode of action evidence was provided recently with observations that developmental Pb exposure of BALB/c mice (100 µM Pb acetate in drinking water of dams from GD8 to PND21) upregulated splenic gene expression of caspase-12 (Kasten-Jolly et al., 2010). Caspase-12 is a cysteine protease that functions in apoptosis and activation of pro-inflammatory cytokines and has been linked with a role in the inhibition of bacterial clearance both systemically and in the gut mucosa (Saleh et al., 2006).

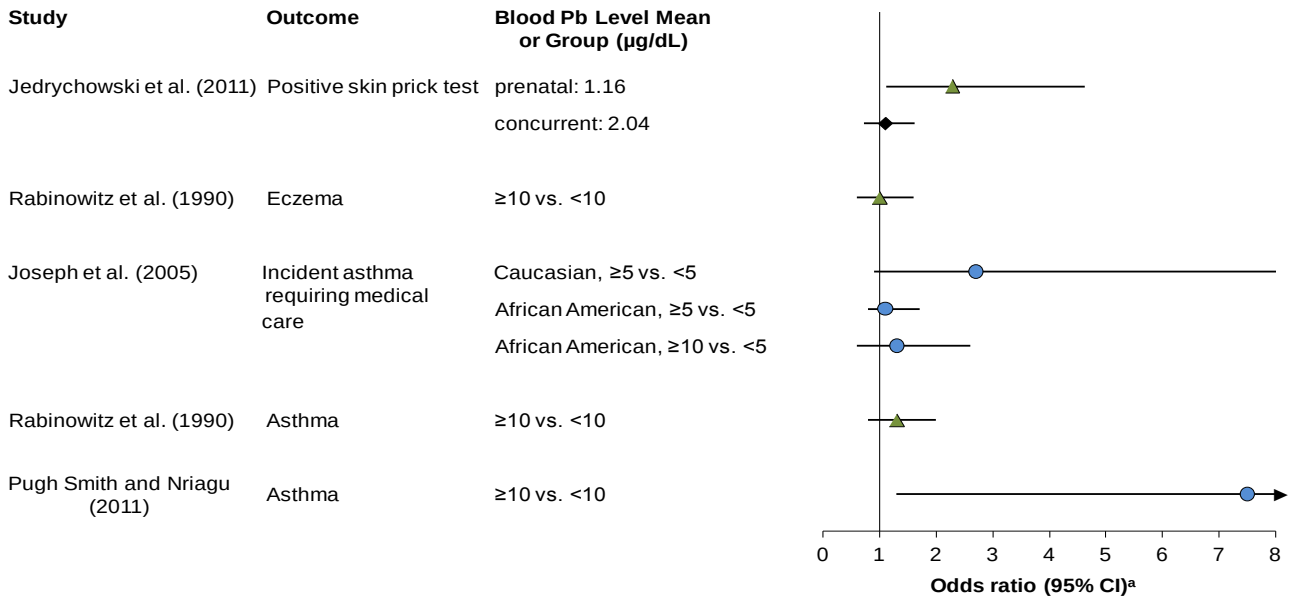
In the few available epidemiologic studies, various Pb exposure indicators (i.e., cord or concurrent blood Pb, Pb content in total deposition samples or lichen) were associated with viral and bacterial infections in children. An increase in infections was associated with cord blood Pb levels ≥ 10 µg/dL in children in Boston, MA (n = 283) (Rabinowitz et al., 1990) and a mean concurrent blood Pb level of 3.34 µg/dL in children in Germany (n = 311) (Karmaus et al., 2005). Similarly, a study found higher frequency of self-reported colds or influenza among 66 Pb battery or smelter plant workers with blood Pb levels 21.3-85.2 µg/dL than among 53 controls with blood Pb levels 6.6-20.8 µg/dL (Ewers et al., 1982). Because of the many limitations, including the lack of consideration for potential confounding (Karmaus et al., 2005; Rabinowitz et al., 1990; Ewers et al., 1982), lack statistical rigor in comparisons of mean blood Pb levels by number of infections (Karmaus et al., 2005; Ewers et al., 1982), and ecological design (Carreras et al., 2009), conclusions about the effects of Pb exposure on viral or bacterial infections cannot rely on epidemiologic evidence and are based primarily on animal evidence. The weak epidemiologic data do not detract from the consistent findings in animals for Pb-induced decreased host resistance, including those in animals with relevant blood Pb levels, and evidence for modes of action including decreased macrophage function and Th1 cytokine production.

With few studies available, the effect of Pb on resistance to eukaryotic parasites is not clear. High concentration Pb acetate (≥ 30 µM) diminished the ability of macrophages to kill *Leishmania enrietti* protozoa parasites in vitro (Mauël et al., 1989). Survival of malaria-infected mice was enhanced with 100 µM Pb nitrate exposure via drinking water

(Koka et al., 2007), which was attributed to Pb inducing eryptosis and removal of infected erythrocytes and not to Pb-induced alterations in immune function. Nriagu et al. (2008) found that higher concurrent blood Pb level was associated with lower malaria prevalence among 653 children (ages 2-9 years) from three Nigerian cities with a mean blood Pb level of 8.9 µg/dL. Results were adjusted for age, sex, number of siblings, and comorbidities such as depressed mood, headaches, and irritability. Given the well-characterized effect of Pb in promoting Th2 activity, it is plausible for Pb to enhance host resistance to parasites that require robust Th2 responses such as helminths. However, this relationship has not been well examined.

4.6.5.2 Atopic and Inflammatory Conditions

Toxicological evidence and to a lesser extent, epidemiologic evidence, have supported the effects of Pb exposure on stimulating Th2 activity, including increasing production of Th2 cytokines such as IL-4 (Section 4.6.6.1), IgE antibody (Section 4.6.3), and inflammation (Section 4.6.4). These endpoints comprise a well-recognized mode of action for the development and exacerbation of atopic and inflammatory conditions such as asthma and allergy. Thus, this mechanistic evidence provides support for the small body of epidemiologic evidence indicating associations of blood Pb levels with asthma or allergy in children (Figure 4-34 and Table 4-33). Whereas such evidence reviewed in the 2006 Pb AQCD was too sparse to permit conclusions, findings from recent studies add supporting evidence. Children examined in studies of asthma and allergy encompassed a wide age range (i.e., <1-12 years) and across studies, blood Pb was measured at different lifestages and ages. Studies ascertained outcomes with parent report of doctor diagnosis but also more objectively using a surveillance database or clinical testing. This variability could contribute to between-study heterogeneity in results; however, the objective assessment of outcomes in some studies is not likely to produce a spurious association. Further, some of the evidence was provided by large studies that prospectively ascertained outcome incidence after the measurement of blood Pb levels, did not indicate selection bias, and considered potential confounding by SES and other environmental exposures. These strengths reduce the likelihood of reverse causation and the influence of other risk factors and increase confidence that the observed associations reflect a direct relationship with Pb exposure.



Note: Results are presented first for allergy-related outcomes then for asthma. All results are from prospective analyses, except for Pugh Smith and Nriagu (2011). Black diamond represents associations with concurrent blood Pb levels, green triangles represent associations with prenatal (cord) blood Pb levels, and blue circles represent associations with blood Pb levels measured in childhood up to 12 months prior to outcome assessment. Odds ratios in Jedrychowski et al. (2011) are standardized to a 1 µg/dL increase in blood Pb level.

Figure 4-34 Associations of blood Pb levels with asthma and allergy in children.

Table 4-33 Additional characteristics and quantitative results for studies presented in Figure 4-34.

Study	Study Population and Methodological Details	Blood Pb Level Data (µg/dL)	Outcome	Odds Ratio or Relative Risk (95% CI)
Jedrychowski et al. (2011)	224 children followed prenatally to age 5 yr, Krakow, Poland Prospective. Study of multiple exposures and outcomes. Clinical assessment of atopy. No information on follow-up participation but no selective attrition. Excluded subjects with cord blood level > 2.5 µg/dL. Logistic regression adjusted for sex, parity, maternal age, education, and atopy, cord blood cotinine, smoker in home during follow-up. Also considered potential confounding by breastfeeding and allergen levels in house dust.	Prenatal (cord) geometric mean: 1.16 (95% CI: 1.12, 1.22) Concurrent geometric mean: 2.04 (95% CI: 1.95, 2.12)	Positive Skin Prick Test	2.3 (1.1, 4.6)[a] 1.1 (0.7, 1.6)[a]
Rabinowitz et al. (1990)	159 children followed from birth to unspecified age, Boston area, MA Prospective. Low participation among eligibles. No information on differences with nonparticipants. Logistic regression with parental report of eczema. No consideration of potential confounding factors.	Prenatal (cord) ≥ 10 vs. <10 Mean not reported, approximate 90th percentile = 10	Eczema	1.0 (0.6, 1.6)[b]
Hon et al. (2010; 2009)[c]	110 children with atopic dermatitis, mean (SD) age: 9.9 (4.6) yr, Hong Kong, China, Not clear whether subjects were free of atopic dermatitis at time of blood Pb measurement. Recruitment from dermatology clinic. Clinical assessment of atopic dermatitis. No information on participation rate. Examination of multiple metals. Lack of rigorous statistical methods. No consideration of potential confounding factors.	Serum Pb mean (SD): 1.86 (0.83)	Atopic dermatitis severity	r = 0.329, p <0.001

Table 4-33 (Continued): Additional characteristics and quantitative results for studies presented in Figure 4-34.

Study	Study Population and Methodological Details	Blood Pb Level Data (µg/dL)	Outcome	Odds Ratio or Relative Risk (95% CI)
Joseph et al. (2005)	4,634 children, ages 4 mo-3 yr followed for 12 months, Southeastern MI Prospective. Large sample size. Indirect assessment of asthma diagnosis but ascertained from managed care organization claims database. Logistic regression adjusted for sex, birth weight, and average annual income available only at census block level. Lack of information on other environmental exposures.	Caucasian, ≥ 5 vs. <5 African American, ≥ 5 vs. <5 African American, ≥ 10 vs. <5 Measured up to 12 mo before outcome Mean (SD) Caucasian: 3.2 (2.5) African American: 5.5 (4.3)	Incident asthma requiring medical care	2.7 (0.9, 8.1)[d] 1.1 (0.8, 1.7)[d] 1.3 (0.6, 2.6)[d]
Rabinowitz et al. (1990)	204 children followed from birth to unspecified age, Boston area, MA Same methodology as that described for eczema above. Parental report of asthma.	Prenatal (cord) ≥ 10 vs. <10 Mean not reported, approximate 90th percentile = 10	Prevalent asthma	1.3 (0.8, 2.0)[b]
Pugh Smith and Nriagu (2011)	356 children, ages 0-12 yr (71% age < 6 yr), Saginaw, MI Cross-sectional. Recruitment from state blood Pb database. Moderate participation rate. Parental report of asthma diagnosis. Logistic regression adjusted for age, sex, family income, #stories in unit, cat in home, dog in home, cockroach problem, # persons in home, smoker in home, clutter, highest blood Pb level at address, candles or incense, months of residency, housing tenure, cooking stove type, main heating source, air conditioning type, peeling paint, ceiling/wall damage, housing age, water dampness/mildew/musty odor.	Blood Pb level ≥ 10 vs. <10 Levels ascertained from statewide database, specific timing unreported but varied among subjects	Prevalent asthma diagnosed within previous 12 months	7.5 (1.3, 42.9)[b]

Note: Results are presented first for allergy-related outcomes then for asthma. Within each category, results are grouped according to prospective or cross-sectional study design.

[a]Odds ratio presented per 1 µg/dL increase in blood Pb level.

[b]Odds ratio in children with blood Pb level ≥ 10 µg/dL with children with blood Pb level <10 µg/dL serving as the reference group.

[c]Results are not included in Figure 4-34 because only correlations are presented.

[d]Relative risk in each specified group with children of the same race with blood Pb level <5 µg/dL serving as the reference group.

Key evidence for Pb-associated effects on allergy-related outcomes was provided by a prospective study in 224 children in Poland that compared associations of prenatal (cord and maternal) and concurrent blood Pb levels with incidence of allergic sensitization at age 5 years, as ascertained by skin prick test (SPT) (Jedrychowski et al., 2011). Subjects assessed at age 5 years did not differ from the full cohort, indicating lack of selective attrition of subjects by blood Pb level or health status. The potential for selection bias specifically by Pb exposure also is reduced because multiple exposures and outcomes were examined in this cohort. A 1 µg/dL increase in prenatal cord blood level was associated with greater risk of positive skin SPT (rash/inflammatory reaction) to dust mite, dog, or cat allergen with an RR of 2.3 (95% CI: 1.1, 4.6). Concurrent blood Pb level was only weakly associated with positive SPT (Figure 4-34 and Table 4-33). For prenatal Pb biomarkers, similar effect estimates were obtained before and after adjustment for sex, parity, maternal age, education, and atopy, and prenatal (cord blood cotinine) and postnatal (smoker in the home) smoking exposure. Results were not altered by additional adjustment for house dust allergen levels. Cord and concurrent blood Pb levels were weakly correlated (r = 0.29), providing support for an independent association for prenatal Pb biomarkers. A relationship with Pb was substantiated by observations that indicators of other exposures, including blood levels of Hg, polycyclic aromatic hydrocarbon DNA adducts, and residential levels of dust mite or pet allergen were associated with lower risks of SPT than was blood Pb level. These associations were observed with relatively low cord blood Pb levels (geometric mean: 1.16 µg/dL [95% CI: 0.12, 1.22], detection limit not reported). However, cord blood Pb levels reflect the pregnancy blood Pb levels of mothers. Because adult blood Pb levels are influenced by both recent and past Pb exposures, there is uncertainty regarding the specific Pb exposure scenarios that contributed to associations between cord blood Pb level and allergic sensitization in children examined in Jedrychowski et al. (2011).

Contrasting results were produced by prospective studies of eczema or atopic dermatitis, which are reactions of the skin to sensitized allergens; however, neither study considered potential confounding factors. Rabinowitz et al. (1990) found no elevated risk of parental reported eczema in children (n = 159) in the Boston, MA area with cord blood Pb levels ≥ 10 µg/dL. Hon et al. (2010; 2009) found a correlation between concurrent serum Pb levels (mean: 1.86 µg/dL, detection limit not reported) and clinically assessed atopic dermatitis severity (e.g., skin area affected, intensity of rash and inflammation, symptoms) in 110 children (mean age 10 years) in Hong Kong (Spearman r = 0.33, p <0.005). Various other metals that were examined were negatively correlated with atopic dermatitis. Although Hon et al. (2010; 2009) examined incidence of atopic dermatitis, the subjects were patients referred to a dermatology clinic. The representativeness of the children to the source population is uncertain, and allergy may already have developed by the time serum Pb levels were measured.

Prospective and cross-sectional evidence indicated associations of blood Pb levels with asthma in children. Among prospective studies, Joseph et al. (2005) accounted for potential confounding factors. Asthma-free children, ages 1-3 years, (N = 4,634) all members of the same managed care organization in southeastern Michigan were selected based on availability of blood Pb level data in a statewide database and then tracked for the following 12 months for incidence of asthma. Incident asthma requiring a doctor visit or medication was defined from the medical claims database as ≥4 asthma medication dispensing events and ≥1 asthma emergency department visit, ≥1 hospitalizations, or ≥4 outpatient visits with at least two asthma medication dispensing events in the previous 12 months. While this definition is not a direct diagnosis, it is used to define persistent asthma by the Healthcare Effectiveness Data and Information Set, which most U.S. health plans use to measure health care performance. The records-based analysis precluded bias due to selective participation of subjects by blood Pb level and health status. However, because a blood Pb measurement was required, it is uncertain whether the study population is representative of the managed care organization population. In analyses that adjusted for average annual income at the census block level, birth weight, and sex, an elevated risk of incident asthma requiring a doctor visit or medication was associated with blood Pb levels ≥ 5 µg/dL in Caucasian children (RR: 2.7 [95% CI: 0.9, 8.1] compared with Caucasian children with blood Pb levels <5 µg/dL) (Figure 4-34 and Table 4-33). In analyses restricted to African Americans, children with blood Pb levels ≥ 10 µg/dL had an elevated risk of incident asthma requiring medical care (RR: 1.3 [95% CI: 0.6, 2.6] compared with children with blood Pb level <5 µg/dL); however the association was imprecise. There were small numbers of children with asthma requiring medical care in the higher blood Pb level categories, which could have accounted for the wide 95% CIs (5 Caucasian children with blood Pb ≥ 5 µg/dL and 9 African American children with blood Pb level ≥ 10 µg/dL). In analyses that used Caucasian children with blood Pb level <5 µg/dL as the reference group, blood Pb level was associated with increased risk of asthma requiring medical care among African American children in all blood Pb level categories, which indicated a stronger association with race. Nonetheless, results within the Caucasian group pointed to an association with blood Pb level. The prospective study of 204 children in the Boston, MA area also found a Pb-associated increased risk of parental-reported asthma (age of assessment not reported), specifically in children with cord blood Pb levels >10 µg/dL relative to those with cord blood Pb levels ≤ 10 µg/dL (Rabinowitz et al., 1990) (Figure 4-34 and Table 4-33). However, potential confounding factors were not examined.

Supporting the prospective evidence, a cross-sectional study conducted in Saginaw, MI found a higher prevalence of parental report of doctor-diagnosed asthma in children (71% ages ≤ 6 years) with blood Pb levels ≥ 10 µg/dL (Pugh Smith and Nriagu, 2011). Similar to Joseph et al. (2005), the study population was predominately African American (78%

of 356). Children were randomly selected from a statewide database of initial blood Pb measurements collected at unspecified ages, and a positive bias is possible if parents of children with higher blood Pb levels and asthma were more likely to participate or recall an asthma diagnosis. Data were collected on asthma diagnosis in the previous 12 months; thus, for some subjects, blood Pb measurement likely preceded asthma diagnosis. A strength of this study was the adjustment for a large number of potential confounding factors such as age, sex, household pets, housing characteristics, household smoking, and family income. Compared with children with initial blood Pb levels <10 µg/dL, children with initial blood Pb levels ≥ 10 µg/dL had a higher odds of having a doctor diagnosis of asthma within the past 12 months (OR: 7.5 [95% CI: 1.3, 42.9]). The results were imprecise, and while this study had more children with blood Pb levels ≥ 10 µg/dL (18.6%) than did Joseph et al. (2005), the analyses considered a much larger number of covariates, some of which may be predictors of Pb exposure.

As discussed in Section 4.6.3.1, one may speculate that cross-sectional associations with asthma and allergy could be attributed to reverse causation. Humans with asthma and animal models of asthma have been shown to have epithelial cell damage and exudation of cells and fluids into airways, which are signs of increased lung permeability. With lung permeability, one may speculate the potential for greater uptake of Pb from airways into blood. Some but not all evidence indicates greater lung clearance of very small particles (^{99m}Tc-DTPA) or uptake into the blood of subjects with asthma than healthy subjects (Del Donno et al., 1997; Rees et al., 1985; O'Byrne et al., 1984; Elwood et al., 1983) and also with airway inflammation associated with fibrosis, smoking, and pulmonary edema [2009 PM ISA (U.S. EPA, 2009a)]. The available evidence does not inform Pb absorption with asthma and allergy directly but provides some indication for higher inflammation resulting in increased lung permeability to very fine particles. However, a role for reverse causation may apply mostly to fine particle Pb and exposure via inhalation, which is not found to be a significant route of exposure (Section 3.1). A relationship between IgE-mediated inflammation and gastrointestinal permeability, a more dominant route of Pb exposure, has not been indicated. These lines of evidence combined with that from prospective studies in children and that characterizing modes of action (i.e., increases in IgE, Th2 cytokines, and inflammation) do not suggest that the associations observed for blood Pb level with asthma and allergy in children are due primarily to reverse causation.

Among the studies in children that found associations of blood Pb level with asthma and allergy, several adjusted for potential confounding by indicators of SES and other environmental exposures. Joseph et al. (2005) adjusted for census block annual income, which may represent family-level income with error. However, Pugh Smith and Nriagu (2011), which examined a primarily low SES population of children in Michigan,

adjusted for family annual income. In this study, a Pb-associated higher asthma prevalence was found with adjustment for various additional factors associated with SES and allergen exposure, including multiple indices of housing condition and presence of pets and cockroaches in the home. Jedrychowski et al. (2011) adjusted for maternal education, and found similar magnitudes of association between cord blood Pb level and positive SPT as those in the unadjusted analysis. Further, residential levels of dust mite or pet allergen were associated with lower risks of SPT than was blood Pb level. Blood Pb level also was associated with asthma or allergy after adjusting for concurrent exposure to smoking in the home (Jedrychowski et al., 2011; Pugh Smith and Nriagu, 2011), with Jedrychowski et al. (2011) additionally adjusting for prenatal smoke exposure assessed as cord blood cotinine levels. The studies varied in the specific confounding factors considered, the method of measurement, and the method of control. Some studies examined multiple potential confounding factors (Jedrychowski et al., 2011; Pugh Smith and Nriagu, 2011), which increases confidence that the associations observed with asthma and allergy, reflect a relationship with Pb. However, in the small evidence base, uncertainty remains regarding residual confounding, particularly by SES. While there is no single complete measure of SES, these studies adjusted for different indicators of SES that may vary in the adequacy of control for confounding. Residual confounding also is possible by factors not examined.

Cross-sectional evidence did not strongly indicate associations of biomarkers of Pb exposure with asthma or allergy in adults (Mendy et al., 2012; Pizent et al., 2008; Bener et al., 2001a). However, study limitations make the evidence less informative for drawing conclusions about the effects of Pb on asthma compared with evidence in children. Higher Pb level in a spot urine sample (geometric mean: 0.59 µg/g creatinine, detection limit not reported) was not associated with an increase in asthma prevalence (OR: 0.72 [95% CI: 0.46, 1.12] per 1 µg Pb/g creatinine increase in urine) in the large U.S. NHANES 2007-2008 analysis of 1,857 adults ages 20 years and older (Mendy et al., 2012). Associations were not found with other respiratory conditions such as emphysema or chronic bronchitis either. This study adjusted for several potential confounding factors: sex, race/ethnicity, education, income to poverty ratio, number of alcoholic drinks/day, and current smoking status. However, spot urinary Pb level has an uncertain relationship with long-term Pb exposure (Section 3.3.3). In a study of adult (ages 19-67 years) office workers in Zagreb, Croatia, Pizent et al. (2008) found a concurrent blood Pb-associated lower odds of positive SPT to common inhaled allergens among 50 men (median blood Pb level: 3.2 µg/dL) and lack of a statistically significant association among 166 women (median blood Pb level: 2.2 µg/dL), adjusting for age, smoking (current and history), and number of alcoholic drinks/day. The findings in women appeared to be discordant because there was an association between concurrent blood Pb level and serum IgE, which commonly mediates the acute inflammatory response to allergens. However, the

interpretation of the findings is difficult because only statistically significant effect estimates were reported; thus, it is not known whether odds ratios were in the same direction for SPT and IgE in women. Bener et al. (2001a) found higher prevalence of asthma and allergy-related conditions in 110 Pb industrial workers (mean age: 35.5 years) than in 110 age-matched controls. However, the implications are limited because blood Pb levels in both Pb-exposed workers and controls (geometric means: 77.5 and 19.8 µg/dL, respectively) were higher than those in most of the current U.S. adult general population, and potential confounding factors, including other occupational exposures, were not evaluated.

In summary, studies support associations of higher blood Pb levels with asthma and allergy in children (Jedrychowski et al., 2011; Pugh Smith and Nriagu, 2011; Joseph et al., 2005). Because of study limitations in the Pb biomarker analyzed and confounding considered, the evidence in adults is largely uninformative (Mendy et al., 2012; Pizent et al., 2008; Bener et al., 2001a). In children, evidence was limited to a few populations. Because of the heterogeneity in the relatively small body of evidence, it was difficult to identify whether the strength of association with asthma and allergy differed by age of children, lifestage or time period of blood Pb measurement (prenatal, sometime in childhood prior to outcome assessment, concurrent), or blood Pb level. The few prospective analyses examining incidence of allergy or asthma (Jedrychowski et al., 2011; Joseph et al., 2005) do not suggest that the observed associations are due to reverse causation. Some but not all evidence links asthma and other inflammatory conditions with increased lung permeability to very small particles, but evidence is lacking for a relationship with increased gastrointestinal permeability, the more dominant ingestion route of Pb exposure. These various lines of evidence indicate that associations observed between blood Pb level and asthma and allergy in children are not attributable primarily to reverse causation. In the epidemiologic studies, the lack of selective participation of subjects and objective assessment of outcomes indicates lack of biased reporting of asthma and allergy for children with higher blood Pb levels. Although not affecting internal validity, in some studies, the recruitment of subjects from blood Pb surveillance databases may limit generalizability of findings. The adjustment for maternal education and exposure to smoking or allergens in Jedrychowski et al. (2011) and family income, smoking, housing conditions, pets, or pests in Pugh Smith and Nriagu (2011) increase confidence that the associations observed in these studies are not solely due to confounding by SES, smoking, or allergen exposure. Further, biological plausibility is well supported by evidence describing modes of action for asthma and allergy, including Pb-associated increases in IgE (Section 4.6.3), Th2 cytokines (Section 4.6.6.1), and inflammation (Section 4.6.4). However, because there are few studies of asthma and allergy, uncertainty remains regarding residual confounding, particularly by SES, which was examined with different indicators across studies.

4.6.5.3 Other Respiratory Effects

Potential relationships between Pb exposure and respiratory effects such as bronchial responsiveness (BR), pulmonary function or morphology, and respiratory symptoms were not well characterized in the 2006 Pb AQCD (U.S. EPA, 2006b) but have been examined recently in a small number of studies. Because these outcomes are not specific to atopic and inflammatory conditions, they are evaluated separately here and in Section 4.9.6. Associations between blood Pb level and indicators of lung function in adults are inconsistent. Studies were cross-sectional, included similarly aged subjects, and considered similar confounding factors. Increased BR is a characteristic feature of asthma and other respiratory diseases and can result from the activation of innate immune responses and increased airway inflammation. In the larger study of 523 adults (ages 19-58 years) in Seoul, Korea, Min et al. (2008a) found an association between concurrent blood Pb level and BR. A 1 µg/dL higher concurrent blood Pb level was associated with a higher BR index (log [(% decline in forced expiratory volume in 1 second [FEV_1]/log of final methacholine concentration in mg/dL)+10]) of 0.018 (95% CI: 0.004, 0.03), with adjustment for age, sex, height, smoking status, baseline FEV_1, and presence of asthma (Min et al., 2008a). The concurrent blood Pb levels in these adults were low (mean [SD]: 2.90 [1.59] µg/dL); however, it is uncertain what timing, level, frequency, and duration of Pb exposures contributed to the observed association. In contrast to Min et al. (2008a), Pizent et al. (2008) found that higher concurrent blood Pb level was associated with lower BR in 47 men (decrease of 2.4 [95% CI: 0.52, 4.2] in log of percentage change in FEV_1 post-histamine challenge/cumulative dose histamine per log increase in blood Pb level adjusted for age and serum Se). Smoking intensity and alcohol consumption were excluded as covariates by stepwise regression. Similarly, among these men, higher blood Pb level was associated with lower odds of positive SPT.

Pb-associated respiratory effects were not clearly indicated in adults with occupational Pb exposures either. However, the lack of direct analysis of blood Pb levels and consideration for potential confounding limit the utility of this evidence in drawing conclusion about the respiratory effects of Pb. In bus drivers (mean age: 46 years) in Hong Kong (Jones et al., 2008; Jones et al., 2006), 129 drivers of non-air conditioned buses had lower exposures to PM_{10}, lower blood Pb levels (mean 3.7 µg/dL versus 5.0 µg/dL in air conditioned buses) but lower indices of lung function than did 358 drivers of air conditioned buses (Jones et al., 2006). The authors attributed the slightly higher blood Pb levels of air conditioned bus drivers to the poor efficiency in the filters resulting in higher PM_{10} levels on those buses. Blood Pb levels and various lung function parameters were similar between 33 roadside vendors and 31 adjacent shopkeepers (respectively, mean ages: 45.1 and 42.8 years and mean blood Pb levels: 5.61 and 5.14 µg/dL) (Jones et al., 2008). Pb industrial workers (N = 100, mean age: 34.6 years) in

the United Arab Emirates had higher prevalence of respiratory symptoms such as cough, phlegm, shortness of breath, and wheeze than did 100 age- and sex-matched unexposed controls (Bener et al., 2001a). Blood Pb levels in both the Pb industrial workers and the control group (geometric means: 77.5 and 19.8 µg/dL, respectively) were higher than those in most of the current U.S. adult general population.

An effect of Pb specifically on the lung was demonstrated in a recent study of Wistar rats with low blood Pb levels (2.1 µg/dL) but produced by Pb acetate given by injection (25 mg/kg, 3 consecutive days) (Kaczynska et al., 2011). The lungs of Pb-treated rats exhibited pulmonary fibrosis, epithelial cell damage, an increase in mast cells, increased recruitment of monocytes and thrombocytes into capillaries, and increased macrophage accumulation in the alveolar space. While these pulmonary changes have been linked with functional pulmonary decrements and inflammation in other studies (unrelated to Pb exposure), the implications are uncertain because the results were obtained with a route of Pb exposure less relevant to those in humans.

Air-Pb Studies

The 2006 Pb AQCD (U.S. EPA, 2006b) did not review respiratory effects studies that represented Pb exposure by Pb measured in ambient air samples. However, there are studies that have examined relationships between respiratory effects and airborne Pb by analyzing the Pb component of PM_{10} or $PM_{2.5}$ air samples individually or as part of a group of correlated components within these samples using source apportionment or principal component analysis. Daily ambient air Pb-PM concentrations were associated with daily respiratory morbidity in children (Gent et al., 2009; Hong et al., 2007b). Gent et al. (2009) found that increases in Pb-$PM_{2.5}$ (same-day or 3-day average) were associated with increases in respiratory symptoms and asthma medication use in 149 children with asthma in Southern New England (ages 4-12 years), adjusting for season, day of the week, and date. Hong et al. (2007b) found that an increase in previous-day Pb-PM_{10} was associated with a decrease in lung function in 43 mostly healthy children in Korea, adjusting for age, sex, height, weight, household smoking, and weather. Toxicological studies found Pb-containing CAPs to induce pulmonary inflammation. Uzu et al. (2011) found that Pb-rich PM from a Pb recycling plant increased the release of the cytokine granulocyte-macrophage colony-stimulating factor from human epithelial cells. Pulmonary inflammation was found in animals exposed to CAPs in which Pb was one of numerous components (Wei et al., 2011; Duvall et al., 2008; Godleski et al., 2002; Saldiva et al., 2002).

As with blood Pb, ambient air Pb-$PM_{2.5}$ concentrations were not consistently associated with respiratory effects in older adults with adjustment for weather and temporal trends.

Among adults ages 65 years and older in 6 California counties, an increase in Pb-$PM_{2.5}$ was associated with an elevated risk of respiratory mortality 3 days later in an all-year analysis (RR: 1.01 [95% CI: 0.99, 1.03] per 4 ng/m^3 increase in Pb-$PM_{2.5}$) and summer-only analysis (RR not reported) (Ostro et al., 2007). However, among adults ages 65 years and older in 106 U.S. counties, Bell et al. (2009) found that an increase in same-day Pb-$PM_{2.5}$ was associated with a decrease in respiratory hospital admissions.

Despite limited available results consistent with a relationship between respiratory effects in children and short-term (over a few days) changes in ambient air Pb-PM concentrations, uncertainties limit the utility of these findings in evaluating Pb-associated respiratory effects. Few data on size distribution of Pb-PM are available, so it is difficult to assess the representativeness of these concentrations to population exposure (Section 2.5.3). Moreover, few data are available on the relationship between blood Pb and air Pb for the varying Pb-PM size distributions (see Section 3.5.1). In several air-Pb studies, other PM components such as elemental carbon, copper, and zinc also were associated with respiratory effects. In the absence of data on correlations among PM components, measurements on co-occurring ambient pollutants, or results adjusted for copollutants, it is difficult to exclude confounding by ambient air exposures to other PM components or ambient pollutants. In several studies that analyzed PM component mixtures, of which Pb-PM comprised one component, it is not possible to attribute the observed associations or lack of associations specifically to Pb (Sarnat et al., 2008; Andersen et al., 2007; Veranth et al., 2006; Maciejczyk and Chen, 2005).

In summary, while a few studies found air Pb-PM to be associated with respiratory effects in children, limitations of these recent results include potential confounding by other air pollutants and the uncertain representativeness of Pb-PM to population exposures. In adults, neither blood Pb nor air Pb-PM was consistently associated with respiratory effects. Blood Pb studies of nonoccupationally-exposed adults were similar in cross-sectional design, age of subjects, potential confounding factors examined, and examination of respiratory endpoints that can exhibit short-term changes. Studies of Pb-exposed workers were similarly limited by lack of rigorous statistical analysis with blood Pb levels and lack of consideration for potential confounding factors, including other occupational exposures.

4.6.5.4 Autoimmunity

Autoimmunity is an immune response against self (e.g., generation of antibodies against self antigens) and is linked with diseases such as lupus and rheumatoid arthritis. Evidence for the effects of Pb on increasing the risk of autoimmunity is provided largely by animal

studies reviewed in the 2006 Pb AQCD in which pre- or post-natal Pb acetate treatment, most by injection, was associated with the generation of auto-antibodies in juvenile and adult animals (Hudson et al., 2003; Bunn et al., 2000; El-Fawal et al., 1999; Waterman et al., 1994). El-Fawal et al. (1999) found elevated auto-antibodies in F344 rats with blood Pb levels 11-50 µg/dL. Results did not indicate whether the mechanism involved a shift toward Th2 or Th1 responses. While recent studies did not examine production of auto-antibodies by Pb, some provided indirect evidence by showing the potential of Pb to induce formation of neo-antigens which in turn could induce the formation of auto-antibodies. For example, Kasten-Jolly et al. (2010) found that developmental Pb acetate exposure of BALB/c mice (100 µM in drinking water, GD8-PND21) upregulated genes for digestive and catabolizing enzymes, which could lead to the generation of self-peptides, which combined with other Pb-induced immune effects, had the potential to induce generation of auto-antibodies. The potential for auto-antibody generation also was indicated by the activation of neo-antigen-specific T cells in adult BALB/c mice injected once with 25-50 µg Pb chloride (Carey et al., 2006). Evidence of Pb-associated autoimmune responses in humans is limited to findings of higher levels of IgM and IgG auto-antibodies to neural proteins in male battery-plant workers (n = 20, 56) with blood Pb levels 10 to 40 µg/dL compared with controls (n = 7, 15, blood Pb levels not reported) (El-Fawal et al., 1999). Pb workers and controls were matched by demographic and SES characteristics, but potential confounding by other workplace exposures was not examined. Consistent with findings in Pb-exposed workers, modified neural proteins were found in CBA/J rats injected with native protein altered by Pb acetate in vitro (Waterman et al., 1994).

4.6.5.5 Tumors

Toxicological evidence indicates that high concentration Pb exposures directly promote tumor formation or induce mutagenesis and genotoxicity and is evaluated in detail in Section 4.10. Kerkvliet and Baecher-Steppan (1982) provided evidence for involvement of the immune system. Postnatal exposure of 6-8 week old male C57BL/6 mice to 130 and 1,300 ppm Pb acetate in drinking water for 10-12 weeks transiently enhanced moloney sarcoma virus-induced tumor growth compared with control animals but did not prevent subsequent tumor regression. The Pb-induced tumor growth was accompanied by impaired macrophage phagocytosis (indicating suppressed Th1 responses) but not cytotoxicity. Cancer promotion is a relatively common outcome in chemical-induced immunotoxicology, particularly when early life exposures are involved (Dietert, 2011).

4.6.6 Modes of Action for Pb Immune Effects

4.6.6.1 Cytokine Production

As referenced in preceding sections, cytokines are signaling molecules that affect immune cell function. For example, IL-4 induces B cells into IgE-producing cells, and IFN-γ induces macrophage recruitment and antigen presenting activity. The 2006 Pb AQCD (U.S. EPA, 2006b) presented a large body of evidence that clearly demonstrated that pre- or post-natal Pb exposure of animals such as rodents and chickens suppressed the production of Th1 cytokine IFN-γ and/or increased production of Th2 cytokines such as IL-4 [Table 5-7 of the 2006 Pb AQCD (U.S. EPA, 2006h)]. The combined evidence for Pb-induced cytokine changes in multiple cell types, including T cells and macrophages, indicates a shift of acquired immunity responses away from Th1 responses and toward Th2 responses. In turn, the Th1 to Th2 shift provides mode of action support for the associations of Pb with downstream events (Figure 4-34) such as inflammation, ROS production, impaired macrophage function, decreased host resistance observed primarily in toxicological studies, increased IgE production observed in both epidemiologic and toxicological studies, and asthma and allergy observed in epidemiologic studies. Previous toxicological studies found Pb to affect cytokine production via action on T cells and macrophages, and a recent study provided new evidence that Th2 skewing may be mediated via effects on dendritic cells.

Many studies found a shift to Th2 cytokine production in animals with long-term (>4 weeks) dietary Pb exposure, and in some studies, the effects of prenatal exposure on cytokine production persisted to the adult lifestage (Chen et al., 2004; Miller et al., 1998). In the few studies that measured blood Pb levels shortly after cessation of Pb exposure (gestational plus postnatal or postnatal only), higher IL-4 and/or lower IFN- γ were found in rodents with relevant blood Pb levels, means 6.75 and 17 µg/dL (Chen et al., 2004; Dyatlov and Lawrence, 2002). Some studies found an increase in IL-4 or decrease in IFN-γ concomitantly with an increase in IgE (Heo et al., 1997; 1996) or decrease in host resistance (Fernandez-Cabezudo et al., 2007) further supporting changes in cytokine production as a mode of action for Pb-induced effects on downstream immune endpoints. A recent study found a shift to Th2 cytokine production in mice over a wide range of Pb exposures and provided evidence of effects at lower Pb exposures and a concentration-dependent relationship. In this study, lifetime (gestation through adulthood) exposure of Swiss mice to 0.06-400 ppm dietary Pb acetate produced blood Pb levels (upon termination of exposure) of 1.23 to 61.48 µg/dL (Iavicoli et al., 2006b). For IL-2 and IL-4, nonlinear concentration-response relationships were found, with the largest decrease and increase, respectively, found between animals with blood Pb levels of

0.8 µg/dL (0.02 ppm Pb acetate, controls) and 1.23 µg/dL. A linear concentration-dependent decrease in IFN-γ was observed in animals with blood Pb levels 0.8-61.48 µg/dL. Although examined in few in vivo studies, increases in IL-6 and IL-10, also Th2 cytokines, were reported in juvenile and adult rodents exposed to Pb gestationally or postnatally in drinking water [Table 5-7 of the 2006 Pb AQCD (U.S. EPA, 2006h)].

In vitro studies also reported a Pb-induced shift to production of Th2 cytokines. In concordance with other indicators of Th2 skewing (i.e., suppressed DTH) in BALB/c mice treated with Pb-exposed dendritic cells, Gao et al. (2007) observed that 10-day 25 µM Pb chloride exposure lowered the ratio of IL-12:IL-10 production by dendritic cells in vitro. Pb did not affect dendritic cell production of IL-6, IL-10, or TNF-α; however, in co-cultures of Pb-treated dendritic cells and T cells, most results indicated that dendritic cells stimulated T cells to produce Th2 cytokines. For example, although T cell production of the Th1 cytokine IL-2 increased, production of Th2 cytokines, IL-6 and IL-10 increased. Further, Pb-treated dendritic cells increased IL-4 production in OVA-specific T cells, indicating that Pb affected the antigen presenting cell function of dendritic cells. In another in vitro study, 24-hour Pb acetate exposures of 0.15 µg/dL and higher suppressed expression of Th1 cytokines, IFN-γ, IL-1β, and TNF-α, and increased secretion of Th2 cytokines, IL-5, IL-6, and IL-10 in cultures of human PMNs activated with *Salmonella enteritidis* or with monoclonal antibodies of CD3, CD28, and CD40, (Hemdan et al., 2005).

Several toxicological studies found Pb-induced increases in the cytokine TNF-α, in some cases, specifically from macrophages (Khan et al., 2011; Cheng et al., 2006; Flohé et al., 2002; Zelikoff et al., 1993). This provides mode of action support for toxicological evidence indicating Pb-induced decreases in resistance to bacterial infection since TNF-α is produced primarily by activated macrophages, is increased in response to infection, and induces inflammation. Among the in vivo studies, increases in TNF-α were found with prenatal dietary Pb acetate exposure (250 ppm) of F344 rats (Chen et al., 1999; Miller et al., 1998), postnatal Pb oxide air exposure (31 µg/m^3, 3 hours/day, 4 days) of rabbits (Zelikoff et al., 1993), and postnatal i.p. Pb acetate treatment (5.0 mg) of Swiss mice (Dentener et al., 1989). The effects of prenatal dietary Pb exposure were found to persist to adulthood. In animals, the Pb-induced increases in TNF-α were accompanied by functional changes in host responses such as decreased macrophage phagocytosis (Zelikoff et al., 1993), suppressed DTH (Miller et al., 1998), and increased mortality to *E.coli* endotoxin (Dentener et al., 1989). Blood Pb levels of animals were infrequently reported; however, Chen et al. (1999) found increased TNF-α in rats with embryonic blood Pb levels of 149 µg/dL. In addition to finding Pb-induced increases in TNF-α (Khan et al., 2011; Gao et al., 2007; Cheng et al., 2006; Flohé et al., 2002; Krocova et al.,

2000; Guo et al., 1996a), some in vitro studies provided mechanistic explanation by finding that Pb acetate or chloride (10-50 µM, 1.5 hours-10 days) induced phosphorylation of mitogen-activated protein kinase (MAPK) signaling molecules (Khan et al., 2011; Gao et al., 2007; Cheng et al., 2006). Further, Cheng et al. (2006) found that blocking protein kinase C or MAPK reduced TNF-α production by macrophages in vitro, which in turn, protected against Pb acetate + LPS-induced liver injury in A/J mice.

The few available epidemiologic studies that examined cytokines found higher concurrent blood Pb levels in children and adults to be associated with higher Th2 cytokine and lower Th1 cytokine levels in serum. The epidemiologic evidence overall was based on cross-sectional analyses, which precludes identifying the temporal sequence between Pb exposure and cytokine changes. Other limitations include the lack of rigorous statistical analysis and limited consideration of potential confounding. Because of these limitations, the epidemiologic evidence is not a primary consideration in drawing conclusions about Pb-associated cytokine changes. However, it does not detract from the compelling toxicological evidence. Among children ages 9 months to 6 years in Missouri recruited from a public assistance or Pb poisoning prevention program, Lutz et al. (1999) found that 8 children with concurrent blood Pb levels 15-19 µg/dL had 4-5 fold higher serum levels of IL-4 ($p = 0.08$, Kruskal Wallis) and 3-fold higher IgE (Section 4.6.3) than did 90 children with lower blood Pb levels. IL-4 levels in 9 children with blood Pb levels 20-44 µg/dL were lower than those in 90 children with blood Pb levels <15 µg/dL. The elevated IL-4 and IgE in children with blood Pb levels 15-19 µg/dL were consistent with the action of IL-4 to activate B cells to induce class switching to IgE. In another study of 214 children in grades 5 and 6 in Taiwan, investigators compared cytokine levels not by blood Pb level groups but by potential for Pb exposures due to age of home and location of residence (Hsiao et al., 2011). Elevated concurrent blood Pb levels were found only among 64 children living near an oil refinery, in particular, among 34 children with known respiratory allergies (mean: 8.8 µg/dL, versus 3.2-3.8 µg/dL in urban and rural groups). Children with allergies near the oil refinery also had the lowest serum levels of IFN-γ (45-fold) and highest levels of IL-4 (6-fold) ($p <0.05$ for comparisons with any subgroup). While the results suggested that residence near the oil refinery contributed to differences in cytokine levels between healthy and allergic children, they do not identify an independent effect of Pb, other exposures or co-occurring factors, or a combination of factors.

Evidence of an association between blood Pb levels and cytokine levels in adults is unclear. However, a relatively large study that considered potential confounding found an association. Sufficient information was not reported to assess potential selection bias. Among 300 (93% male, mean age 24 years) healthy university students in Incheon, Korea, higher concurrent blood Pb level was associated with higher serum levels of TNF-

α and IL-6, adjusting for age, BMI, and smoking status (Kim et al., 2007). Associations were larger in magnitude among the 147 males in the upper two quartiles of blood Pb levels, 2.51-10.47 µg/dL. Associations with these blood Pb levels may reflect contributions of higher past Pb exposures. A 1 µg/dL higher blood Pb level was associated with a 0.75 (95% CI: 0.14, 1.36) pg/mL higher TNF-α and a 0.18 (95% CI: -0.02, 0.38) pg/mL higher IL-6. The association between levels of blood Pb and plasma TNF-α was greater among men who were GSTM1 null (n = 77) than men who were GSTM1 positive and men who had the TNF-α GG genotype (n = 131) than men who had the GA or AA genotype. For the association between blood Pb level and plasma IL-6, the effect estimate was slightly elevated in TNF-α GG genotype but similar between GSTM1 genotypes. In this study, there were multiple comparisons, but a consistent pattern of association was observed across the immune endpoints examined. Subgroup analyses are prone to higher probability of findings by chance, but these analyses had fairly large sample sizes, and some results had biological plausibility. Pb has been shown to increase ROS (Section 4.2.4), and cytokine expression has been shown to be modulated by ROS-sensitive transcription factors. Thus, it is biologically plausible that the null variant of GSTM1, which is associated with reduced elimination of ROS, may be associated with increased cytokine levels. The results for the TNF-α variant are difficult to interpret. The GG genotype is associated with lower expression of TNF-α, but the literature is mixed with respect to which variant increases risk of inflammation-related conditions.

Much smaller studies of adults that did not consider potential confounding, did not report quantitative results but only reported lack of statistically significant correlations between concurrent blood Pb level and serum Th2 and Th1 cytokine levels in men (n = 17 with and 17 without allergy, ages 19-52 years, median blood Pb levels: ~11 µg/dL) (Boscolo et al., 1999) and women in Italy (n = 23 with and 25 without allergy, ages 19-49 years, median blood Pb levels: 6.4 and 5.5 µg/dL, respectively) (Boscolo et al., 2000).

Results from studies of occupationally-exposed adults also suggested that Pb exposure may be associated with decreases in Th1 cytokines and increases in Th2 cytokines (Di Lorenzo et al., 2007; Valentino et al., 2007; Yücesoy et al., 1997a). Valentino et al. (2007) had the most rigorous statistical methods comprising regression analyses with adjustment for age, BMI, smoking status, and alcohol consumption status but not other occupational exposures. Regression coefficients describing the concentration-response functions were not reported; however, 44 male foundry workers in Italy (mean blood Pb levels: 21.7 µg/dL) and 14 pottery workers (mean blood Pb level: 9.7 µg/dL, ages of all workers 30-61 years) had higher plasma IL-10 (ANOVA, $p < 0.05$) than did the 59 unexposed controls (mean blood Pb level: 3.9 µg/dL, ages 25-61 years). Levels of Th2 cytokines IL-2, IL-6, and IL-10 also increased from the lowest to highest blood Pb group (ANOVA, $p > 0.05$). In contrast with most other studies, both exposed worker groups had

lower IL-4 levels compared with controls (ANOVA, p >0.05). In Yücesoy et al. (1997a), serum levels of the Th1 cytokines, IL-1β and IFN-γ, were lower in 20 Pb-exposed workers (mean blood Pb level: 59.4 µg/dL, ages 19-49 years) than in the 12 age-matched controls in Turkey (mean blood Pb level: 4.8 µg/dL). Some (Di Lorenzo et al., 2007; Valentino et al., 2007) but not all (Yücesoy et al., 1997a) studies found higher serum TNF-α in Pb-exposed workers. DiLorenzo et al. (2007) found a monotonic increase from the 28 unexposed (blood Pb levels not reported, mean age: 48.2 years) to 17 intermediate worker (blood Pb: 9.1-29.4 µg/dL) and 19 high worker (blood Pb: 29.4-81.6 µg/dL) blood Pb level groups (mean age of Pb-exposed workers: 45.3 years) in Italy. Results also indicated a potential interaction between blood Pb level and smoking. Among current smokers (n = 9 to 20), a 13- to 16-fold difference in TNF-α levels was observed among blood Pb groups. Among nonsmokers (n = 2 to 8), the differences were less than two fold.

In summary, the few epidemiologic studies indicate associations of higher concurrent blood Pb level with higher levels of IL-4 and/or lower levels of IFN-γ in children (Hsiao et al., 2011; Lutz et al., 1999) and occupationally-exposed adults (Di Lorenzo et al., 2007; Valentino et al., 2007; Yücesoy et al., 1997a). Because quantitative results were not reported in each study of nonoccupationally-exposed adults, implications of findings are difficult to assess. Limitations of the epidemiologic evidence overall include the cross-sectional design of studies and lack of rigorous statistical analysis that considered potential confounding factors. Sufficient data were not reported to assess potential selection bias. Because of the many limitations, the epidemiologic evidence alone is not used to draw conclusions about Pb-associated cytokine changes. However, they are useful in supporting the relevance of toxicological evidence to humans. Biological plausibility for an effect of Pb on cytokine production is provided by a large body of toxicological evidence that clearly demonstrates a Pb-induced shift to a Th2 phenotype with increases in the Th2 cytokine IL-4 and decreases in the Th1 cytokine IFN-γ. Several of these observations were made in juvenile and adult animals exposed prenatally or postnatally via diet that resulted in blood Pb levels (upon cessation of Pb exposure) relevant to this ISA, 1.23-17 µg/dL (Iavicoli et al., 2006b; Chen et al., 2004; Dyatlov and Lawrence, 2002). While results were not uniform for other cytokines (i.e., Th1 cytokine IL-2), most available results pointed to increases in Th2 cytokines, specifically, IL-6 and IL-10, in Pb-exposed animals. Results from Gao et al. (2007) pointed to a role for dendritic cells in skewing T cells to Th2 cytokine production. Several studies demonstrated Pb-induced increases in TNF-α but in animals with high prenatal dietary or postnatal air Pb exposure or blood Pb levels (e.g., 149 µg/dL) (Chen et al., 1999; Miller et al., 1998; Zelikoff et al., 1993). In vitro evidence indicates that Pb may induce increases in TNF-α via MAPK signaling pathways (Khan et al., 2011; Gao et al., 2007;

Cheng et al., 2006). Animal evidence indicates effects on cytokine production by prenatal and postnatal Pb exposures, whereas epidemiologic studies examined only concurrent blood Pb level. Because concurrent blood Pb level in children and adults reflects both previous and recent Pb exposures, associations with concurrent blood Pb level may reflect an effect of cumulative Pb exposure. Overall, the consistent toxicological evidence for Pb-induced decreases in Th1 cytokines and increases in Th2 cytokines and pro-inflammatory cytokines such as TNF-α provides clear mode of action support for the evidence indicating the effects of Pb on increases in IgE, inflammation, atopic and inflammatory conditions, and decreases in host resistance.

4.6.6.2 Decreased Nitric Oxide

As described in the 2006 Pb AQCD (U.S. EPA, 2006b), key mode of action support for the effects of Pb on impairing macrophage function and decreasing host resistance was provided by consistent toxicological findings for Pb-induced decreases in NO, which is involved in the cytotoxic activity of macrophages in host defense processes [see 2006 Pb AQCD Annex Table AX5.9.6 (U.S. EPA, 2006i)]. In adult rodents, decreases in NO from macrophages were observed with short-term Pb acetate exposures (1 or 6 days) during early gestation (Bunn et al., 2001b; Lee et al., 2001b) but not long-term exposures occurring during the full gestational period (Bunn et al., 2001c; Chen et al., 1999; Miller et al., 1998). With short-term exposure, decreases in NO were found in Sprague-Dawley rats with blood Pb level 4.5 µg/dL in males and 5.3 µg/dL in females measured 2 weeks after Pb exposure in drinking water of dams was terminated (Bunn et al., 2001b) and in chicks with blood Pb levels that did not exceed 11 µg/dL but with Pb injected into eggs embryos (Lee et al., 2001b).

The short-term in vivo findings are supported by several in vitro observations of decreased release of NO from macrophages and splenocytes induced by a wide range of Pb exposure concentrations (0.625-5 µM) and durations (2 hours-6 days) (Farrer et al., 2008; Mishra et al., 2006a; Krocova et al., 2000; Chen et al., 1997; Tian and Lawrence, 1996, 1995). Farrer et al. (2008), with 5 µM Pb glutamate, further indicated that the mode of action for Pb may involve a decrease in inducible NO synthase function in myeloid cells without a change in its gene expression. Also in this study, Pb abrogated the myeloid cell (CD11b+)-mediated suppression of CD4+ T cell proliferation, and exogenous NO restored suppression. Together, these findings indicated that Pb may indirectly enhance T cell proliferation through its effect on decreasing NO production. Combined with the observation that Pb can alter antigen processing (Farrer et al., 2005) and, hence, the quality and magnitude of the acquired immune response against pathogen exposure, evidence indicated that multiple arms of host defense against infectious

challenge can be compromised. Diminished production of NO in innate immune cells such as macrophages could affect other physiological systems (e.g., neurological, cardiovascular, endocrine) that require NO signaling cascades.

Consistent with the toxicological evidence, cross-sectional studies found associations between concurrent blood Pb level with lower NO in populations living near Pb sources (Barbosa et al., 2006c; Pineda-Zavaleta et al., 2004). These studies did not report sufficient information on participation rates; however, examination of populations near Pb sources could limit generalizability of findings. Additional limitations include the cross-sectional design, which impedes determination of the temporal sequence between Pb exposure and NO suppression and the limited consideration for potential confounding. Because of these limitations, epidemiologic evidence is not a major consideration in drawing conclusions about the effects of Pb on NO. However, it is useful in that it shows Pb-associated decreases in NO in humans, similar to toxicological studies.

In a study of 65 children (ages 6-11 years) in Region Lagunera, Mexico, mean concurrent blood Pb levels increased (7.02 to 20.6 to 30.38 µg/dL) with increasing school proximity (8,100-650 meters) to a Pb smelter (Pineda-Zavaleta et al., 2004). With adjustment for age and sex, a 1 µg/dL higher blood Pb level was associated with a 0.00089 (95% CI: -0.0017, -0.00005) log lower NO release from macrophages activated by phytohemagglutinin (PHA). Because PHA activates macrophages indirectly through the activation of lymphocytes, the results indicated that Pb suppressed T cell-mediated macrophage activation. Blood Pb group comparisons indicated that associations were due largely to the lower NO in the 23 children closest to the smelter who had blood Pb levels 10.31-47.49 µg/dL. Though not described in detail, higher blood Pb level was not associated with lower NO in girls.

Among 104 adults (ages 18-60 years) in Sao Paolo, Brazil residing near a closed battery plant, Barbosa et al. (2006c) observed an association between higher concurrent blood Pb level and lower plasma NO in the 69 adults (mean blood Pb level: 6.4 µg/dL) with the TC or CC eNOS genotype ($r = 0.23$, $p = 0.048$). The results are consistent with the reduced promoter activity and potentially reduced gene expression of the TC/CC variants. Results were not adjusted for potential confounding factors, but subjects were nonsmoking, nonalcohol drinking with normal mean BMI and SBP. The inclusion criteria may further limit the generalizability of findings. Because NO was measured in plasma, immune cells could not be identified as the source of NO. In contrast, Valentino et al. (2007) found similar plasma NO levels in 44 male foundry workers (mean blood Pb level: 21.7 µg/dL), 14 male pottery workers (mean blood Pb level: 9.7 µg/dL), and 59 male unexposed workers of similar age (mean blood Pb level: 3.9 µg/dL, ages 25-61 years). Quantitative

results were not reported, but blood Pb level was reported not to be correlated with NO. Potential confounding factors, including other workplace exposures were not examined.

In summary, short-term dietary Pb exposure early in gestation but not long-term exposure for the full gestational period was found to reduce NO production by macrophages in adult animals. In vitro evidence consistently demonstrates Pb-induced decreases in NO from macrophages and splenocytes. The relevance of toxicological evidence is supported by observations of an association between higher concurrent blood Pb level and lower release of NO from macrophages of children in Mexico. The association in children was due largely to lower NO in macrophages from children living near a Pb smelter with concurrent blood Pb levels >10 µg/dL, higher than those in most of the current U.S. population of children. This study was cross-sectional and had limited consideration for potential confounding factors. However, the toxicological evidence provides clear mode of action support for the effects of Pb on decreasing host resistance given the role of NO in mediating the cytotoxic activity of macrophages.

4.6.6.3 Increased Reactive Oxygen Species and Prostaglandins

ROS are released from macrophages during phagocytosis and are involved in killing invading bacteria. ROS and PGE_2 are important mediators of inflammation which can result in local tissue damage (Figure 4-33). The roles of ROS and PGE_2 in both host defense and injury may explain some of the inconsistencies in the evidence as reported in the 2006 Pb AQCD. In activated macrophages undergoing phagocytosis, high concentration (10-1,000 µM, 15 minutes-20 hours) Pb chloride or acetate exposures were found to reduce release of ROS (Hilbertz et al., 1986; Castranova et al., 1980), consistent with observations of Pb-induced decreased resistance to bacterial or viral infection. Also in stimulated macrophages, Hilbertz et al. (1986) found an increase in ROS with 1-hour Pb chloride exposure but a decrease in ROS (and phagocytosis) with 20-hour exposure indicating differential responses by exposure duration. Similarly, Shabani and Rabbani (2000) found 3-hour Pb nitrate exposure (240 µM) to increase ROS from alveolar macrophages and induce their apoptosis, consistent with impaired host defense. Also similar to Hilbertz et al. (1986), Chen et al. (1997) found 18-hour Pb glutamate exposure (4 µM) to induce a decrease in ROS but did not indicate the functional state of macrophages. Other studies reported reductions in antioxidants such as glutathione and catalase in conjunction with reduced macrophage function in Swiss mice treated with Pb nitrate by oral gavage (40 mg/kg per day, 30 days) (Lodi et al., 2011) or increases in PGE_2 and apoptosis in cultures of neuroblastoma cells with 0.01-10 µM Pb nitrate (3 hours) (Chetty et al., 2005). While several processes have been proposed to explain the

mechanisms of Pb-induced oxidative damage, the exact combination of processes involved remains to be determined (Section 4.2.4).

In adult animals, Pb exposure increased ROS release from macrophages immediately upon cessation of exposure (Baykov et al., 1996; Zelikoff et al., 1993) but not 9-10 weeks after exposure (Miller et al., 1998), consistent with in vitro findings. These Pb exposures occurred through relevant routes of exposure, i.e., diet or air, but with high concentrations, e.g., 31 µg/m^3 Pb oxide in air for 3 hours/day for 4 days in rabbits (Zelikoff et al., 1993) and 1.5 mg/kg Pb acetate in diet for 30 days in Swiss mice (Baykov et al., 1996). Neither study measured the blood Pb levels of animals.

Pb-associated increases in ROS also were found in macrophages of children. However, the findings are based on a cross-sectional analysis with limited consideration for potential confounding and higher blood Pb levels (means: 7.02, 20.6, and 30.38 µg/dL) than most of those in the current U.S. population. In addition to finding suppressed NO production (Section 4.6.6.2), Pineda-Zavaleta et al. (2004) found a Pb-associated increase in ROS production from macrophages in children in Mexico living in varying proximities to a Pb smelter. With adjustment for age and sex, a 1 µg/dL higher concurrent blood Pb level was associated with a 0.00389 (95% CI: 0.00031, 0.00748) log higher release of superoxide anion from macrophages directly activated by IFN-γ/LPS. The blood Pb-associated superoxide anion release was larger from macrophages of males. Because IFN-γ directly activates macrophages, these results indicated that Pb stimulated cytokine-induced macrophage activation. Blood Pb level was not associated with ROS from neutrophils in a study of male Pb recycling workers (ages 19-45 years) in India. Despite large differences in blood Pb levels between 30 Pb workers (mean: 106 µg/dL) and 27 unexposed controls (mean: 4.5 µg/dL), levels of ROS released from neutrophils (indicators of respiratory burst) were similar between groups (Mishra et al., 2006a). However, evidence does not clearly indicate that neutrophils are a major responding cell to Pb exposure (Section 4.6.2.5).

PGE_2 is produced from the metabolism of cell membrane phospholipids and may be released by macrophages to modulate their function in a paracrine or autocrine manner. An increase in PGE_2 from macrophages was found in turkeys exposed to Pb acetate in feed during the early postnatal period (PND1-PND21) which produced a high blood Pb level, i.e., mean of 42 µg/dL (Knowles and Donaldson, 1997). However, dietary Pb acetate exposure (250-2,000 ppm) of chicks during the same developmental period (PND1-PND19) resulted in an increase in serum arachidonic acid but not PGE_2 or other prostaglandins (Knowles and Donaldson, 1990). In vitro studies also used high Pb exposure concentrations, >20 µM Pb chloride (Flohé et al., 2002; Lee and Battles, 1994). A recent in vitro study with human neuroblastoma cells found increases in PGE_2 with

lower Pb concentrations (0.01-1 µM Pb acetate) than previously reported (Chetty et al., 2005).

In summary, ROS and PGE_2 function in modulating macrophage function, aiding in bacterial killing, and inducing tissue damage as part of an inflammatory response. Consistent with these diverse roles, toxicological studies have found both Pb-associated increases and decreases in ROS and PGE_2. Consistent with findings in animal and in vitro models, a cross-sectional study found an association between higher concurrent blood Pb level (>10 µg/dL) and higher ROS release from macrophages in children in Mexico with adjustment for potential cofounding only by age and sex. In animals, Pb-induced increases in macrophage production of ROS and PGE_2 occurred concomitantly with functional alterations such as impaired macrophage phagocytosis and apoptosis. Toxicological results were based on examination of high Pb concentrations but are considered to be relevant in the ISA for informing mode of action. The toxicological observations of Pb-induced effects on ROS and PGE_2 provide evidence for modes of action underlying the effects of Pb on reduced macrophage function and decreased host resistance.

4.6.6.4 Cellular Death (Apoptosis, Necrosis)

The 2006 Pb AQCD reported contrasting effects of Pb on the apoptosis of macrophages in vitro (U.S. EPA, 2006b). However, with a few recent studies in mice, evidence suggests that Pb exposure may induce cell death of immune cells via apoptosis or necrosis. Xu et al. (2008) found that 4-week dietary exposure of juvenile ICR mice to Pb acetate (50-100 mg/kg) induced DNA damage in peripheral blood lymphocytes, increased p53 and Bax expression in the liver, but did not change Bcl-2 expression (creating a Bax/Bcl-2 imbalance). Bax promotes apoptosis, whereas Bcl-2 inhibits apoptosis. Concomitant increases in indicators of oxidative stress in liver homogenate suggested that oxidative stress may mediate the effects of Pb in promoting apoptosis. In Swiss mice, Bishayi and Sengupta (2006) found splenic macrophages to have elevated DNA fragmentation, a key event in apoptosis, but with i.p. Pb acetate treatment (10 mg/kg). Consistent with in vivo findings, Gargioni et al. (2006) found 20 and 40 µM Pb nitrate to induce cell death in mouse peritoneal macrophages in vitro which occurred concomitantly with a loss of cell membrane integrity, indicating that Pb primarily induced macrophage necrosis or cell lysis. While evidence for Pb-induced cell death of immune cells with routes of Pb exposure relevant to this ISA is sparse, the evidence suggests that the induction of cell death may be a potential mode of action by which Pb affects macrophage function and decreases host resistance.

4.6.7 Immune Effects of Pb within Mixtures

In the 2006 Pb AQCD (U.S. EPA, 2006b), the immune effects resulting from Pb within metal mixtures were not well characterized; however with recent studies, there is some evidence for greater immune effects resulting from co-exposure to Pb and other metals. In Swiss mice treated with Pb acetate (10 mg/kg i.p., daily for 15 days), As (0.5 mg/kg i.p., daily for 15 days), or both, Bishayi and Sengupta (2006) reported a greater than additive effect of co-administered Pb and As on decreasing bacterial resistance, decreasing macrophage myeloperoxidase release, and NO production. Epidemiologic studies have not widely examined interactions between Pb and other metals. However, consistent with Bishayi and Sengupta (2006), Pineda-Zavaleta et al. (2004) (Section 4.6.6.2) found interactions between Pb and As among 65 children in Mexico ages 6-11 years. Contamination of drinking water by both Pb and As was a concern in the study area; however, urinary As levels were higher in children who had lower blood Pb levels. Higher urinary As was associated with lower NO release from macrophages (similar to blood Pb). Higher internal dose of both As and Pb was associated with a larger decrease in NO (p for interaction = 0.037) than was dose of either metal alone. A positive interaction (p = 0.042) between blood Pb and urinary As was found for superoxide anion release from indirectly activated macrophages. But each metal alone was significantly associated with superoxide anion release only from directly activated macrophages. Because of the high blood Pb levels in these children (means in three groups at varying distances from a Pb smelter: 7, 20.6, 30.4 µg/dL), it is not clear whether these relationships would apply to the current U.S. population of children.

Institoris et al. (2006) found that Cd or Hg co-exposure potentiated the effects of Pb. Lymph node weight decreased in 4 week-old Wistar rats exposed to 20 mg/kg Pb acetate by drinking water plus another metal but not with Pb alone. In contrast, Fortier et al. (2008) did not find metal co-exposure to increase the effects of Pb. Pb chloride (7.5-20.7 µg/dL) did not alter lymphocyte proliferation, monocyte phagocytosis, or NK cell activity in human leukocyte cultures. A mixture of 20.7 µg/dL Pb chloride plus 12.0 µg/dL methylmercuric chloride (MeHgCl) decreased lymphocyte proliferation; however, these effects were attributed to MeHgCl, which singly had a stronger suppressive effect. Other toxicological studies found metal mixtures that included Pb to decrease antibody titers or increase neutrophil counts (Jadhav et al., 2007; Massadeh et al., 2007) but did not test each metal individually. The latter findings cannot necessarily be attributed to interactions between Pb and other components within the mixture. Overall, several results indicated that exposures to Pb-containing metal mixtures are associated with immune effects. Some but not all results showed greater immune effects with co-exposures to Pb and metals such as As, Cd, or Hg than with Pb exposure alone.

4.6.8 Summary and Causal Determination

The cumulative body of epidemiologic and toxicological evidence describes several effects of Pb exposure on the immune system related to a shift from Th1- to Th2-type responses, including an increase in atopic and inflammatory conditions and a decrease in host resistance. Outcomes related to an increase in atopic and inflammatory conditions include asthma, allergy, increased IgE, and mode of action endpoints such as selective differentiation of Th2 cells, increased production of Th2 cytokines, B cell activation, and increased inflammation. Outcomes related to decreased host resistance include enhanced susceptibility to bacterial and viral infection, suppressed DTH, and those describing mode of action, i.e., decreased production of Th1 cytokines, reduced phagocyte function, and increased inflammation. A small body of studies indicates the effects of Pb exposure on autoimmunity. The sections that follow describe the evaluation of evidence for these three groups of outcomes, increased atopic and inflammatory conditions, decreased host resistance, and autoimmunity, with respect to causal relationships with Pb exposure using the framework described in Table II of the Preamble. The application of key evidence, supporting or contradicting, to the causal framework is summarized in Table 4-34.

4.6.8.1 Evidence for an Increase in Atopic and Inflammatory Conditions

Epidemiologic and toxicological evidence together indicate that a causal relationship is likely to exist between Pb exposure and atopic and inflammatory conditions. This relationship is supported by evidence for associations of blood Pb levels with asthma and allergy in studies in children (Jedrychowski et al., 2011; Pugh Smith and Nriagu, 2011; Joseph et al., 2005), Pb-associated increases in IgE in children and animals, and evidence describing modes of action including increases in Th2 cytokines and inflammation.

Recent studies on asthma and allergy expand upon the evidence presented in the 2006 Pb AQCD by providing additional evidence from prospective analyses, and by better addressing uncertainties regarding potential confounding by factors such as SES and smoking and residential allergen exposures (Table 4-34). Findings from studies that prospectively ascertained outcomes provide support for the directionality of associations (Jedrychowski et al., 2011; Joseph et al., 2005). In these studies, the lack of selective participation and objective assessment of outcomes of asthma and allergy through medical records or clinical testing, respectively, indicates lack of biased reporting of asthma and allergy in children with higher blood Pb levels (Section 4.6.5.2 and Table 4-34). Among children age 5 years in Poland, Jedrychowski et al. (2011) found that a 1 µg/dL increase in prenatal cord blood Pb level was associated with an increased risk of

allergic sensitization of 2.3 (95% CI: 1.1, 4.6). The magnitude of risk did not differ with and without adjustment for maternal education or residential allergen levels. An additional strength of this study was the adjustment for prenatal and postnatal smoking exposure. A large study of 4,634 children in Michigan ages 1-3 years found that compared with Caucasian children with blood Pb levels <5 µg/dL measured up to 12 months before asthma assessment, Caucasian children with blood Pb levels ≥ 5 µg/dL had an increased risk of incident asthma of 2.7 (95% CI: 0.9, 8.1) (Joseph et al., 2005). Adjustment was made for census block average income, which may not adequately control for potential confounding by individual subject-level SES.

Supporting evidence was provided by a cross-sectional study of 356 children (71% ages <6 years) in Michigan, which found that compared with children with concurrent blood Pb levels <10 µg/dL, children with concurrent blood Pb level ≥ 10 µg/dL had increased parental report of an asthma diagnosis in the previous 12 months with an OR of 7.5 (95% CI: 1.3, 42.9) (Pugh Smith and Nriagu, 2011). This study was cross-sectional and produced an imprecise effect estimate; however, a strength of the study was the relatively extensive consideration for potential confounding, including adjustment for family-level income. As with Jedrychowski et al. (2011), Pugh Smith and Nriagu (2011) found an association with adjustment for smoking exposures in the home plus other indicators of housing exposures and condition (Table 4-34). Some covariates may be predictors of Pb exposure and control for Pb exposure itself. The studies of asthma and allergy differed in which and how potential confounding factors were considered, particularly SES. While there is no single complete measure of SES, the heterogeneity in SES indicators used across these few studies produces uncertainty regarding residual confounding. Residual confounding also is possible by factors not examined. The examination of maternal education and exposure to smoking or allergens in Jedrychowski et al. (2011) and family income, smoking, housing conditions, pets, or pests in Pugh Smith and Nriagu (2011) increase confidence in the associations observed for blood Pb levels. However, because evidence is limited to a few populations, there is uncertainty regarding potential confounding of Pb associations by SES and other exposures well characterized in the literature to be associated with asthma and allergy.

With respect to blood Pb levels associated with atopic and inflammatory conditions, Joseph et al. (2005) found elevated incidence of asthma in Caucasian children with earlier childhood blood Pb levels ≥ 5 µg/dL. Pugh Smith and Nriagu (2011) found higher asthma prevalence in children with concurrent blood Pb levels ≥ 10 µg/dL. Jedrychowski et al. (2011) found increased allergic sensitization in association with cord blood Pb levels that were low (geometric mean: 1.16 µg/dL). However, because cord blood Pb levels are influenced by maternal blood Pb levels, the associations may reflect higher past maternal Pb exposures.

Biological plausibility for the relationships found between blood Pb levels and asthma and allergy in children is provided by evidence characterizing modes of action, namely, a Pb-associated shift in production from Th1 cytokines (e.g., IFN-γ) to Th2 cytokines (e.g., IL-4) and increase in Th2-dependent IgE levels (Table 4-34). A majority of this evidence was available in the 2006 Pb AQCD (U.S. EPA, 2006b). The shift from Th1 to Th2 cytokine production in animals was found with prenatal or postnatal (4 weeks in juveniles, 3 weeks or 8 weeks in adults) dietary Pb exposures. In the studies available in humans (Table 4-34), higher concurrent blood Pb levels were associated with higher serum IL-4 in children (Lutz et al., 1999) and higher serum IL-6 in nonoccupationally-exposed adults (adjusted for age, BMI, and current smoking status and additionally adjusted for income, physical activity, education, and history of inflammatory conditions in the large NHANES analysis) (Songdej et al., 2010; Kim et al., 2007). Because of the limitations in the small body of epidemiologic studies, i.e., the cross-sectional design of studies and inconsistent consideration for potential confounding, the epidemiologic evidence is a lesser consideration in drawing conclusions about Pb-associated cytokine changes. However, epidemiologic evidence does not detract from the clear toxicological evidence for Pb-induced increases in Th2 cytokine production. Coherence for a shift from Th1 to Th2 cytokine production is found in the in vitro evidence for Pb-induced selective differentiation of naïve T cells to a Th2 subtype (Heo et al., 1998; 1996; McCabe and Lawrence, 1991). A recent study in adult mice and in vitro provided new evidence that Pb may promote the shift to Th2 responses by increasing production of Th2 cytokines in dendritic cells, the major effector in antigen responses (Gao et al., 2007).

Additional mode of action support is provided by associations observed between higher concurrent blood Pb levels and higher serum IgE in several different populations of children (Section 4.6.3, Table 4-34). While most studies found elevated IgE in groups of children with concurrent blood Pb levels >10 µg/dL, Karmaus et al. (2005) found higher serum IgE in children ages 7-10 years in Germany with blood Pb levels 2.8-3.4 µg/dL compared with children with lower blood Pb levels. A monotonic increase in IgE was found across increasing blood Pb groups, except in the highest group in Lutz et al. (1999) but not in other studies (Hegazy et al., 2011; Karmaus et al., 2005). Lutz et al. (1999) recruited children in Michigan from a public assistance or Pb poisoning prevention program, and Karmaus et al. (2005) recruited schoolchildren but excluded those from homes where more than 12 cigarettes were smoked per day. The nature of recruitment may limit generalizability of findings. Sufficient information was not reported to assess biased participation by Pb exposure and history of allergy or asthma. The limited consideration for potential confounding comprised adjustment for age (Karmaus et al., 2005; Lutz et al., 1999), smoking exposure, serum lipids, blood organochlorine levels, and previous infections (Karmaus et al., 2005) but not SES or allergen exposure. Although these findings were based on cross-sectional analyses and had limited

consideration for potential confounding, they were supported by similar findings in animals, which are not subject to reverse causation or confounding bias. Despite clear evidence in animals overall, there was some inconsistency for Pb-induced increases in IgE in animals with gestational or gestational/lactational dietary Pb exposures that resulted in blood Pb levels 5-20 µg/dL, which are more relevant to this ISA (Chen et al., 2004; Snyder et al., 2000). Miller et al. (1998) found elevated IgE in adult F344 rats after gestational Pb exposure via drinking water of dams whose blood Pb levels peaked at 30-39 µg/dL. There is lack of coherence between the consistent results for IgE and the inconsistent findings for Pb-induced activation of B cells, which differentiate into allergic antibody-producing cells (Section 4.6.3). However, there is strong mode of action support in animals for Pb-induced increases in IL-4, which stimulates differentiation of B cells.

Further support for the effects of Pb exposure on increasing risk of atopic and inflammatory conditions is provided by evidence of Pb-associated inflammation (Section 4.6.4 and Table 4-34). Coherence for this evidence is found with findings for Pb-induced increases in IgE which primes basophils and mast cells to release pro-inflammatory mediators. Pb-induced inflammation is clearly demonstrated by a large toxicological evidence base for the effects of Pb exposure on inducing macrophages into a hyperinflammatory state as characterized by enhanced production of TNF-α, PGE_2, and ROS. Inflammation was observed in rabbits exposed to Pb via air for 4 days (31 $\mu g/m^3$) (Zelikoff et al., 1993) and rodents exposed via diet (250 ppm drinking water during gestation, 1.5 mg/kg food postnatally for 30 days) (Miller et al., 1998; Baykov et al., 1996). Epidemiologic evidence is sparse and therefore is a lesser consideration in drawing conclusions regarding the effects of Pb exposure on inflammation. But, consistent with previous toxicological evidence, a large analysis of adults participating in NHANES found an association in 4,278 men between concurrent blood Pb levels and serum CRP, an indicator of systemic inflammation, with adjustment for age, BMI, income, physical activity, education, history of inflammatory conditions, cardiovascular disease, diabetes, and smoking status (Songdej et al., 2010). Because only concurrent blood Pb levels were examined, there is uncertainty regarding the temporal sequence between Pb exposure and inflammation and the magnitude, timing, frequency, and duration of Pb exposures that contributed to the observed association.

With respect to important lifestages of Pb exposure, gestational or gestational/lactational Pb exposures, producing blood Pb levels 8 µg/dL 2 weeks postweaning and 20 µg/dL at age 1-2 weeks, were found to affect endpoints such as IgE and/or cytokine levels in juvenile and adult rodents (Chen et al., 2004; Snyder et al., 2000). However, increases in Th2 cytokines also were found in adult animals with lifetime Pb exposures beginning in gestation and producing blood Pb levels 1-12 µg/dL upon cessation of exposure (Iavicoli et al., 2006b). The Pb exposure lifestage, magnitude, frequency, and duration associated

with atopic and inflammatory conditions are not well characterized in humans. Cord blood Pb level was associated with allergic sensitization in children (Jedrychowski et al., 2011), whereas other studies of asthma, allergy, IgE, cytokines, and inflammation in children and adults examined blood Pb levels concurrently with outcomes or at some unspecified time before outcome assessment (Table 4-34). In children and adults, concurrent blood Pb levels are influenced by cumulative Pb exposure history (from remodeling of bone) and recent Pb exposures. Thus, the evidence overall indicates that prenatal only or cumulative Pb exposure may influence atopic and inflammatory conditions. However, neither toxicological nor epidemiologic evidence clearly identifies an individual critical lifestage or duration of Pb exposure that is more strongly associated with atopic and inflammatory conditions.

In conclusion, prospective studies in a few populations of children indicate associations of asthma and allergy with blood Pb levels measured prenatally or sometime before the outcome, with a cross-sectional study providing supporting evidence with associations for concurrent blood Pb. Prospective design, lack of selective participation of subjects, and objective assessment of outcomes reduce the likelihood of undue selection bias. These few studies varied in their consideration for potential confounding by SES and exposure to smoking or allergens. Thus, uncertainty remains regarding residual confounding in associations observed between blood Pb levels and asthma and allergy in children. The evidence for asthma and allergy is supported by cross-sectional associations found between higher concurrent blood Pb levels in children and higher IgE, an important mediator of asthma and allergy. The biological plausibility for the effects of Pb on IgE is provided by consistent findings in animals with gestational or gestational-lactational Pb exposures, with some evidence at blood Pb levels relevant to this ISA. In children, higher IgE and higher asthma prevalence were examined and found mostly in groups with concurrent blood Pb levels >10 µg/dL. Coherence for the evidence of Pb-associated increases in asthma, allergy, and IgE is found with evidence for most of the examined endpoints related to mode of action, i.e., Pb-induced increases in Th2 cytokine production and inflammation in animals. The biological plausibility from toxicological evidence, evidence from prospective studies in children, and lack of evidence for inflammation-related permeability in major routes of Pb exposure such as the gastrointestinal system together suggest that reverse causation is not the primary explanation for associations observed between Pb and atopic and inflammatory conditions. Neither toxicological nor epidemiologic evidence clearly identifies an individual critical lifestage or duration of Pb exposure associated with atopic and inflammatory conditions but points to an influence of prenatal only or cumulative Pb exposures. The epidemiologic evidence in a few populations and toxicological evidence together supporting a relationship between Pb exposure and asthma, allergy, and shift to a Th2 phenotype as an underlying mode action but some uncertainty regarding potential confounding is sufficient to conclude that a

causal relationship is likely to exist between Pb exposures and an increase in atopic and inflammatory conditions.

4.6.8.2 Evidence for Decreases in Host Resistance

Evidence indicates that a causal relationship is likely to exist between Pb exposure and decreased host resistance based primarily on consistent observations that relevant Pb exposures decrease responses to antigens (i.e., suppresses DTH) and increase bacterial titers and subsequent mortality in rodents (Table 4-34, Sections 4.6.2.3 and 4.6.5.1). A majority of this evidence was available in the 2006 Pb AQCD (U.S. EPA, 2006b). The studies that reported blood Pb levels demonstrated increased bacterial titers and mortality with adult-only 16 week Pb exposure via drinking water in adult mice with *Salmonella typhimurium* infection and blood Pb level 20 µg/dL (Fernandez-Cabezudo et al., 2007) and with lactational (PND1-PND22) Pb exposure in juvenile mice with *Listeria monocytogenes* infection and blood Pb level 25 µg/dL (Dyatlov and Lawrence, 2002). DTH was suppressed in adult rats with blood Pb levels 6 and 25 µg/dL after gestational Pb exposure in drinking water (Chen et al., 2004; Bunn et al., 2001a). While a few epidemiologic studies found higher prevalence of respiratory infections in children with higher concurrent blood Pb levels (Karmaus et al., 2005; Rabinowitz et al., 1990) and Pb-exposed workers (Ewers et al., 1982), the implications are limited by the lack of rigorous statistical analysis (i.e., regression) and consideration for potential confounding. These limitations also apply to the recent ecologic study finding increased prevalence of child respiratory infections in areas with higher lichen or soil Pb (Carreras et al., 2009) (Table 4-34). These limitations produce uncertainty about the effects of Pb exposure on decreased host resistance in humans but do not detract from the consistent evidence in animals.

The effects of Pb on decreased host resistance are well supported by evidence describing underlying modes of action (Table 4-34). Evidence in animals indicates Pb-induced functional impairment of macrophages, which phagocytize pathogens. Decreased macrophage colony formation was found in rats after gestational Pb exposure (Bunn et al., 2001b), and decreased phagocytic activity was found in mice and turkeys after lactational or 2-week juvenile Pb exposure (Knowles and Donaldson, 1997; Kowolenko et al., 1991). Additional coherence for Pb-induced decreased host resistance is found with observations in animals that gestational Pb exposure suppressed macrophage production of NO which is involved in bacteria killing (Section 4.6.6.2), and postnatal Pb exposure (air for 4 days, food for 30 days) increased production of ROS and PGE_2, which mediate tissue damage (Section 4.6.6.3). Similarly, a cross-sectional epidemiologic study found a smaller release of NO and larger release of superoxide anion from macrophages of

children ages 6-11 years with higher concurrent blood Pb levels (10.3-47.5 versus <10.3 µg/dL) (Pineda-Zavaleta et al., 2004) after adjustment for age and sex. Because of the limited consideration for potential confounding in this study and examination of higher blood Pb levels than those in most current U.S. children, the results are a lesser consideration in drawing conclusions about the effects of Pb on macrophages. However, they do suggest the relevancy of toxicological observations to humans. The killing capability of macrophages is enhanced by the Th1 cytokine IFN-γ. Therefore, an effect of Pb exposure on decreased host resistance is additionally supported by clear evidence in animals for the effects of Pb exposure on suppressing IFN-γ production (Section 4.6.6.1). A recent study in mice indicated that Pb-induced suppression of DTH may be mediated by a shift in production from Th1 to Th2 cytokines specifically in dendritic cells (Gao et al., 2007).

Some evidence did not contribute strong support for the mode of action for Pb-induced decreased host resistance. Pb-exposed workers were found to have reduced functionality of neutrophils, which respond to bacterial infection (Table 4-34, Section 4.6.2.5) but without consideration for potential confounding or analogous toxicological evidence. Neither epidemiologic nor toxicological evidence clearly demonstrated an effect of Pb exposure on NK cells, which respond to viral infection (Section 4.6.2.7).

With respect to important lifestages of Pb exposure, animal studies found that gestational Pb exposures, producing blood Pb levels of 6 and 25 µg/dL, resulted in decreases in Th1 cytokines, suppression of DTH, and greater susceptibility to bacterial infection (Chen et al., 2004; Bunn et al., 2001a). However, these effects related to decreased host resistance also were affected in mice by lactational (Dyatlov and Lawrence, 2002), adult-only (>4 weeks) (Fernandez-Cabezudo et al., 2007), and lifetime gestation to adult Pb exposures (Iavicoli et al., 2006b) that produced blood Pb levels 1-25 µg/dL. Thus, the animal evidence does not clearly identify an individual lifestage or time period of Pb exposure that is more strongly associated with decreased host resistance.

In conclusion, animal toxicological observations are the primary contributors to the evidence for Pb-induced decreased host resistance. Several studies in rodents show that dietary Pb exposures producing relevant blood Pb levels result in increased susceptibility to bacterial infection and suppressed DTH. Further, coherence is found with evidence describing modes of action, including suppressed production of Th1 cytokines and decreased macrophage function in animals. These effects were found with gestational, lactational, adult-only, and lifetime Pb exposures of animals, without an individual critical lifestage of exposure identified. A few cross-sectional epidemiologic studies indicate Pb-associated increases in respiratory infections but limitations, including the lack of rigorous methodology and consideration for potential confounding produce

uncertainty in the epidemiologic evidence for decreased host resistance in humans. The consistent toxicological evidence in animals for increased susceptibility to bacterial infection, suppressed DTH, and related modes of action but uncertainty in the epidemiologic evidence in humans is sufficient to conclude that a causal relationship is likely to exist between Pb exposure and decreased host resistance.

4.6.8.3 Evidence for Autoimmunity

Animal toxicological evidence describes the potential of Pb to increase autoimmunity, with a few previous studies showing Pb-induced generation of auto-antibodies (Hudson et al., 2003; Bunn et al., 2000; El-Fawal et al., 1999; Waterman et al., 1994) and recent studies providing indirect evidence by showing formation of neoantigens that could result in the formation of auto-antibodies (Table 4-34). Several observations were made in animals injected with Pb, which is a route of exposure with uncertain relevance to humans. Higher levels of auto-antibodies also were found in Pb-exposed battery workers; however, implications are limited because of the high blood Pb levels (range: 10-40 µg/dL) of some of the workers and lack of consideration for potential confounding by several factors, including other occupational exposures (El-Fawal et al., 1999). Because the results combined from available toxicological and epidemiologic studies do not sufficiently inform Pb-induced generation of auto-antibodies with relevant Pb exposures, the evidence is inadequate to determine if there is a causal relationship between Pb exposure and autoimmunity.

Table 4-34 Summary of evidence supporting immune causal determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Biomarker Levels Associated with Effects[c]
Increase in Atopic and Inflammatory Conditions – Likely Causal			
Multiple epidemiologic studies show associations with relevant blood Pb levels	A few high-quality prospective studies indicate higher asthma and allergy incidence in association with early childhood or prenatal blood Pb levels in children (ages 1-5 yr) (U.S., Poland)	Joseph et al. (2005), Jedrychowski et al. (2011), Section 4.6.5.2	Groups (ages 1-3 yr) with blood Pb levels measured earlier in childhood >10 µg/dL Prenatal (cord): geometric mean 1.16 µg/dL
	Some studies report high participation and/or follow-up retention, not conditional on Pb exposure or outcome.	Joseph et al. (2005), Jedrychowski et al. (2011)	
	Some studies objectively assessed outcomes with clinical testing, medical records.	Jedrychowski et al. (2011)	
		Pugh Smith and Nriagu (2011)	Group (71% age <6 yr) with concurrent blood Pb levels >10 µg/dL
	Other supporting cross-sectional evidence in children (ages 6 mo-10 yr) for increases in IgE but with limited consideration for potential confounding factors, particularly SES. Associations observed in studies in U.S., Europe, Asia; insufficient information to assess potential selection bias.	Karmaus et al. (2005), Hegazy et al. (2011), Lutz et al. (1999), Hon et al. (2010; 2009), Sun et al. (2003) Section 4.6.3	Groups (ages 6 mo- 10 yr) with concurrent blood Pb levels >10 µg/dL
	Mixed evidence for monotonic C-R varies for IgE. Some studies show increasing IgE across blood Pb groups, except in highest group.	C-R found: Lutz et al. (1999) No C-R: Karmaus et al. (2005), Hegazy et al. (2011)	
But, potential confounding difficult to address	A few studies adjust or consider potential confounding by SES, exposure to smoking, and/or allergen. Heterogeneity in evaluation of potential confounding among the few available studies produces uncertainty.	Jedrychowski et al. (2011), Pugh Smith and Nriagu (2011)	

Table 4-34 (Continued): Summary of evidence supporting immune causal determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Biomarker Levels Associated with Effects[c]
Coherence with toxicological evidence at relevant exposures	Most animal studies show elevated IgE in animals with prenatal and postnatal dietary Pb exposures. Some inconsistency in animals with relevant Pb concentrations.	Increase in IgE: Snyder et al. (2000), Miller et al. (1998)	Increase with gestational-lactational exposure, blood Pb means 5, 20 µg/dL Gestational exposure, maternal blood Pb peak: 30-39 µg/dL
		No increase in IgE: Chen et al. (2004) Section 4.6.3	No increase with gestational Pb exposure, blood Pb means 7-8 µg/dL
Most evidence clearly describes mode of action			
Stimulation of Th2 phenotype	Extensive, consistent evidence of increased production of Th2 cytokines (e.g., IL-4, IL-6, IL-10) in animals with prenatal and postnatal (4 weeks juvenile, 3, 8 weeks adults) dietary Pb exposures. Recent evidence for the role of dendritic cells in mediating Th2 shift.	Section 4.6.6.1 Table 5-7 of the 2006 Pb AQCD (U.S. EPA, 2006h)	
	Limited available cross-sectional evidence in children, adults but with limited consideration for potential confounding. Is not a major consideration in conclusions.	Children: Lutz et al. (1999) Adults: Kim et al. (2007) Section 4.6.6.1	Children (ages 6 mo-6 yr): Concurrent blood Pb group range 15-19 µg/dL Adults: Group range 2.5-10.5 µg/dL
	The few available in vitro studies indicate activation Th2 cells from naïve T cells or over Th1 cells.	Section 4.6.2.1	
Inflammation	Extensive evidence for increased production of TNF-α, IL-6, ROS, PGE_2 by macrophages from animals with prenatal and postnatal (dietary 4 weeks juvenile, dietary 3, 8 weeks adults, air 4 days adults) Pb exposure. Supported by in vitro evidence.	Sections 4.6.6.1 and 4.6.6.3	
	Cross-sectional association observed in children living near Pb source, adjusted for confounding by age and sex but not other factors such as SES. Cross-sectional evidence in adults in NHANES adjusted for inflammatory conditions, smoking and SES. Is not a major consideration in conclusions.	Children: Pineda-Zavaleta et al. (2004) Sections 4.6.6.2, and 4.6.6.3 Adults: Songdej et al. (2010) Section 4.6.4	Children: Group (ages 6-11 yr) with concurrent blood Pb levels >10 µg/dL Adults: Group with concurrent blood Pb levels >1.16 µg/dL

Table 4-34 (Continued): Summary of evidence supporting immune causal determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Biomarker Levels Associated with Effects[c]
Most evidence clearly describes mode of action (continued) B cell activation	Inconsistent toxicological evidence in animals for B cell activation by Pb exposure concentration and duration and strain.	Section 4.6.3	
	Inconsistent epidemiologic evidence for B cell abundance. B cell activation not examined.	Table 4-32 and Section 4.6.3	
Decreases in Host Resistance – Likely Causal			
Multiple animal toxicological studies demonstrate effects with relevant exposures	The few studies with relevant dietary Pb exposures demonstrate increased bacterial infection, sickness behavior, and mortality in mice. Similar observations in several other studies with higher Pb exposures.	Dyatlov and Lawrence (2002), Fernandez-Cabezudo et al. (2007) Section 4.6.5.1	Blood Pb means 20 µg/dL after adult 16-week exposure, 25 µg/dL after lactational exposure
	The few studies with relevant prenatal dietary Pb exposures show suppressed DTH in rodents. Similar observations in several other studies with higher Pb exposures.	Chen et al. (2004), Bunn et al. (2001a) Section 4.6.2.3	Blood Pb means 6.75, 25 µg/dL after gestational exposure
But, epidemiologic evidence is limited and not sufficiently informative	Epidemiologic studies found associations with increased respiratory infections but limitations include lack of consideration for potential confounding or rigorous statistical analysis, and/or ecologic study design	Children: Karmaus et al. (2005), Rabinowitz et al. (1990), Carreras et al. (2009)	Children (ages 7-10 yr): Group with concurrent blood Pb levels >3.34 µg/dL, Group with cord blood Pb levels >10 µg/dL
		Pb-exposed workers: Ewers et al. (1982) Section 4.6.5.1	Pb-exposed workers: Concurrent blood Pb levels 21-85 µg/dL

Table 4-34 (Continued): Summary of evidence supporting immune causal determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Biomarker Levels Associated with Effects[c]
Most evidence clearly describes mode of action			
Decreased macrophage function	Decreased macrophage colony formation in animals with dietary prenatal and postnatal Pb exposure; not widely examined.	Section 4.6.2.4	
	Decreased macrophage phagocytosis in animals and in cell culture, not widely examined.	Section 4.6.2.4	
	Several studies demonstrate decreased NO production by macrophages from animals with prenatal Pb exposure. Supported by in vitro evidence.	Section 4.6.6.2	
	Cross-sectional association of decreased NO in macrophages of children living near Pb source with higher concurrent blood Pb level, adjusted for age and sex but not SES.	Pineda-Zavaleta et al. (2004) Section 4.6.6.2	Children (ages 6-11 yr): Group with concurrent blood Pb levels >10 µg/dL
	Inconsistent evidence in Pb-exposed workers but for macrophage abundance, not function.	Pinkerton et al. (1998), Fischbein et al. (1993), Conterato et al. (2013)	
Decreased Th1 cytokine (IFN-γ) production	Consistent evidence from a large body of toxicological studies with prenatal and postnatal (4 weeks juvenile, 3, 8 weeks adults) dietary Pb exposures of animals.	Section 4.6.6.1 and Table 5-7 of the 2006 Pb AQCD (U.S. EPA, 2006h)	
Decreased neutrophil or NK cell function	Evidence for reduced neutrophil function in Pb-exposed workers but lacks consideration for potential confounding and analogous toxicological evidence.	Section 4.6.2.5	
	Toxicological and epidemiologic evidence does not strongly indicate effects on NK cells.	Section 4.6.2.7	

Table 4-34 (Continued): Summary of evidence supporting immune causal determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Biomarker Levels Associated with Effects[c]
Autoimmunity – Inadequate			
Available toxicological and epidemiologic evidence is of insufficient quantity or quality	A study in rats shows generation of auto-antibodies with relevant adult-only dietary Pb exposure for 4 days. Several other studies have Pb exposure concentrations and/or routes (e.g., i.p.) with uncertain relevance to humans.	Rats: El-Fawal et al. (1999) Section 4.6.5.4	Rats: Blood Pb level range 11-50 µg/dL
	Evidence for increased auto-antibodies in Pb-exposed workers with high blood Pb levels and limited consideration for potential confounding, including other workplace exposures.	Pb-exposed workers: El-Fawal et al. (1999) Section 4.6.5.4	Pb-exposed workers: Blood Pb level range: 10-40 µg/dL

[a]Described in detail in Table II of the Preamble.

[b]Describes the key evidence and references, supporting or contradicting, that contribute most heavily to causal determination. References to earlier sections indicate where full body of evidence is described.

[c]Describes the blood Pb levels in children with which the evidence is substantiated and blood Pb levels in animals most relevant to this ISA.

4.7 Hematological Effects

4.7.1 Introduction

The effects of Pb exposure on red blood cell function and heme synthesis have been extensively studied over several decades in both human and animal studies. The 1978 *National Ambient Air Quality Standard for Lead* was set to protect blood Pb levels in children from exceeding 30 µg/dL as such levels were associated with impaired heme synthesis, evidenced by accumulation of protoporphyrin in erythrocytes (U.S. EPA, 1978).

The 2006 Pb AQCD (U.S. EPA, 2006b) reported that Pb exposure significantly decreases several hematological parameters including hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Further, the 2006 Pb AQCD reported that Pb affects developing red blood cells (RBCs) in children and occupationally exposed adults as noted by anemia observed with concurrent blood Pb >40 µg/dL. Pb-induced anemia is thought to occur due to decreased RBC life span and effects on Hb synthesis. The exact mechanism for these effects is not known, although Pb-induced changes in Fe uptake or inhibition of enzymes in the heme synthetic pathway may be responsible. Once Pb enters the cells, it is predominantly found in protein-bound form, with Hb and aminolevulinic acid dehydratase (ALAD) both identified as targets.

Consistent with the majority of human evidence that high Pb blood levels (i.e., >20 µg/dL in adults) are associated with decreased hematological indices, blood Pb levels >100 µg/dL were associated with decreased RBC survival in laboratory animals. Effects on RBC membrane mobility were observed in rats at blood Pb levels as low as 10 µg/dL, although the precise mechanisms underlying these effects of Pb are not known. Studies conducted in animal and in vitro models provide evidence of multiple other effects on RBC membranes, including altered microviscosity and fluidity, decreased sialic acid content, decreased lamellar organization, decreased lipid resistance to oxidation (possibly mediated by perturbations in RBC membrane lipid profiles), and increased permeability. These alterations to RBC membranes may indicate potential modes of action by which Pb induces RBC fragility, abnormal cellular function, RBC destruction, and ultimately anemic conditions. Pb exposure also has been shown to result in increased activation of RBC scramblase, an enzyme responsible for the expression of phosphatidylserine (PS) on RBC membranes. This expression of PS decreases the life span of RBCs via phagocytosis by macrophages. Pb exposure has been observed to alter the phosphorylation profiles of

membrane proteins, which may influence the activity of membrane enzymes and the functioning of receptors and channels located on the membrane.

The 2006 Pb AQCD reported that Pb exposure affects heme synthesis in humans and animals through the inhibition of multiple key enzymes, most notably ALAD, the enzyme that catalyzes the second, rate-limiting step in heme biosynthesis (Figure 4-35 presents a schematic representation of the heme biosynthetic pathway). The 2006 Pb AQCD (U.S. EPA, 2006b) further reported that decreased RBC ALAD activity is the most sensitive measure of human Pb exposure, in that measurement of ALAD activity is correlated with blood Pb levels. Concentration-response changes in the ratio of activated/nonactivated ALAD activity in avian RBCs were observed to be not dependent on the method of Pb administration. The Pb-associated inhibition of the ALAD enzyme was consistently observed in RBCs from multiple species, including birds, cynomolgus monkeys, and humans. Pb was also observed to inhibit other enzymes responsible for heme biosynthesis, including ferrochelatase, porphobilinogen (PBG) deaminase, and coproporphyrinogen oxidase. Pb also potentially alters heme biosynthesis through inhibition of transferrin (TF) endocytosis and Fe transport.

Pb has been found to alter RBC energy metabolism through inhibition of enzymes involved in anaerobic glycolysis and the pentose phosphate pathway. Pb was also found to inhibit pyrimidine 5'-nucleotidase (P5N) activity, and the 2006 Pb AQCD indicated that this might be another biomarker of Pb exposure. Inhibition of P5N results in an intracellular increase in pyrimidine nucleotides leading to hemolysis and potentially ultimately resulting in anemic conditions. The 2006 Pb AQCD indicated that perturbations in RBC energy metabolism may be related to significant decreases in levels of nucleotide pools, including nicotinamide adenine nucleotide (NAD), possibly due to decreased NAD synthase activity, and nicotinamide adenine nucleotide phosphate (NADP) accompanying significant increases in purine degradation products.

The 2006 Pb AQCD identified oxidative stress as an important potential mode of action by which Pb exposure induced effects on RBCs. Increased lipid peroxidation and inhibition of antioxidant enzymes in RBCs (e.g., superoxide dismutase [SOD], catalase [CAT]) were observed following exposure to Pb.

The epidemiologic and toxicological studies published since the 2006 Pb AQCD, largely support the reported Pb-associated effects on RBC function and heme synthesis. Epidemiologic studies support previous observations that occupationally-exposed adults with higher blood Pb levels than the current U.S. general population (>26 µg/dL) have decreased RBC numbers. However, a few epidemiological studies investigating occupationally-exposed adults and pregnant women provide some evidence that more relevant blood Pb levels, <10 µg/dL, are associated with decreased RBC numbers,

possibly through decreased survival of the RBCs. Effects seen in children are largely consistent with those observed in adults, and a number of toxicological studies support findings observed in human populations. Recent epidemiologic and toxicological studies also support previous findings that Pb biomarker levels or exposure in adults, children, and laboratory animals decreases ALAD activity, as well as the activity of other enzymes in the heme biosynthetic pathway. Recent epidemiologic and toxicological studies expand upon the evidence that Pb exposure results in oxidative stress in RBCs. Although the epidemiologic studies included below are cross-sectional in study design and are limited to observed associations between concurrent blood Pb levels and hematological effects, they do improve upon earlier studies as more studies characterize the effects in children, and they investigate effects in populations with blood Pb levels more comparable to those in the current U.S. population. Additionally, the associations observed in these cross-sectional studies are supported by a large number of animal toxicology studies.

4.7.2 Red Blood Cell Function

As stated in the 2006 Pb AQCD (U.S. EPA, 2006b), Pb poisoning in children has been associated with anemia. As of 2006, the mechanism for this was not clear, but it was determined not to be due to Fe deficiency, which can be found to occur independently of Pb exposure. However, Zimmerman et al. (2006) found that administration of an Fe-fortified diet to non-anemic (or mildly anemic) Fe-deficient Indian children aged 5 to 9 years (baseline median BLL [range] = 11.6 [2.1-24.1] µg/dL) reduced median blood Pb levels to 8.1 µg/dL (range: 3.1-21.9 µg/dL). This reduction in blood Pb levels was statistically significantly greater than in children fed a non-fortified diet for 30 weeks (median blood Pb level [range]: 10.2 [4.4–25.3] µg/dL). Differences in blood Pb levels between diet intervention groups were not different after only 14 weeks. Although a number of epidemiologic studies found decreases in RBCs and/or Hct levels associated with higher blood Pb levels, it is not known whether this is due to reduced RBC survival or a decrease in RBC production. Regardless, decreased RBC survival and hematopoiesis can be expected to occur simultaneously, and any effect on RBC numbers is likely a combination of the two modes of action.

4.7.2.1 Pb Uptake, Binding, and Transport into Red Blood Cells

The 2006 Pb AQCD (U.S. EPA, 2006b) reported that Pb uptake into human RBCs occurs via passive anion transport mechanisms. Although Pb can passively cross the membrane in both directions, little of the Pb is found to leave the cell after entry. Simons (1993b)

found that in vitro uptake of ^{203}Pb (1–10 µM) occurred via an anion exchanger while the efflux occurred via a vanadate-sensitive pathway. After entry into the RBC, radioactive Pb was found to partition with Hb at a ratio estimated to be about 6000:1 bound to unbound (Simons, 1986). However, Bergdahl et al. (1997a) suggested that ALAD was the primary Pb-binding protein and not Hb. The 2006 Pb AQCD also reported that the majority (approximately 98%) of Pb accumulates in RBC cytoplasm bound to protein, and only about 2% is found in the membrane. This is related to the high ratio of Pb in RBCs compared to plasma Pb. Further information on Pb binding and transport in blood can be found in the kinetics section of Chapter 3 (Section 3.2).

Although no recent studies were identified that examined transport of Pb into RBCs, Lind et al. (2009) recently observed that several Zn ionophores (8-hydroxyquinoline derivatives and Zn and Na pyrithione) were able to effectively transport Pb out of RBCs into the extracellular space.

4.7.2.2 Red Blood Cell Survival, Mobility, and Membrane Integrity

A number of cross-sectional studies have investigated the effect of exposure to Pb on various inter-connected and related hematological parameters in children and adults. As these studies were cross-sectional in design, there is uncertainty regarding the directionality of effects and the magnitude, timing, frequency, and duration of Pb exposure that contributed to the observed associations. Additionally, unless explicitly noted, potential confounding by subject characteristics and other workplace or residential exposures was not accounted for in these studies. Adults and children exposed to Pb may also have been co-exposed to other contaminants that can affect the hematological system, and the potential for co-exposure was not assessed in most studies.

In an earlier cross-sectional study of children in Idaho (aged 1-9 years) with blood Pb levels ranging from 11 to 165 µg/dL (approximately 40% were >40 µg/dL), a 10% probability of anemia (Hct <35%) was predicted (in association with blood Pb levels of ~20 µg/dL [age 1 year], 50 µg/dL [age 3 years] and 75 µg/dL [age 5 years]) (Schwartz et al., 1990). More recent studies have also demonstrated negative effects on hematological parameters in children due to the Pb exposure. Ahamed et al. (2006) studied 39 male urban adolescents in India who were separated into groups according to their blood Pb level (Group 1: <10 µg/dL [mean 7.4 µg/dL], Group 2: >10 µg/dL [mean 13.27 µg/dL]). Although the groups were similar in age (mean [SD]: 16.59 [0.91] versus 16.76 [0.90] years, respectively), height, weight, and BMI (therefore, not considered to be potential confounders), Group 2 had a significantly lower packed cell volume (PCV) compared to Group 1. In a related study, Ahamed et al. (2007) investigated the relationship between

blood Pb level, anemia, and other hematological parameters in urban children in India (N = 75). Children were split into two groups as above: Group 1 had blood Pb levels <10 µg/dL (mean [SD]: 6.89 [2.44] µg/dL, n = 19), whereas Group 2 had blood Pb levels >10 µg/dL (mean [SD]: 21.86 [7.58] µg/dL, n = 56). As with the earlier study, ages were similar between the two groups: mean [SD]: 4.68 [1.49] and 4.11 [1.77] years, respectively. Hb and Hct were significantly decreased in Group 2, compared to Group 1 after adjusting for age, sex, and area of residence. Children in Group 2 had an increased odds ratio of anemia (OR: 2.87 [95% CI: 1.60, 2.87]) compared to Group 1 after adjustment for age, sex, and area of residence.

In a cross-sectional study measuring blood Hb as the independent variable, blood Pb levels were observed to decrease with increasing blood Hb. Riddell et al. (2007) found that 21% of children, who were 6 months to 5 years of age living in the rural Philippines, had concurrent blood Pb levels >10 µg/dL (total population mean: 6.9 µg/dL). After controlling for potential confounding by age, sex, birth weight, and history of breastfeeding, Hb levels were inversely related to blood Pb level, with a decrease of 3% blood Pb associated with every 1 g/dL increase in Hb. Among children aged 6-36 months (N = 222) living in Montevideo, Uruguay, 32.9% had blood Pb levels greater than 10 µg/dL (population mean [SD]: 9.0 [6.0] µg/dL) (Queirolo et al., 2010). The mean [SD] Hb concentration was 10.5 [1.5] g/dL, and 44.1% of children were diagnosed as anemic (Hb <10.5 g/dL). Blood Pb levels were significantly higher in anemic children compared to non-anemic (mean [SD]: 10.4 [6.8] versus 7.9 [5.1] µg/dL), and anemic children were more likely to have elevated blood Pb after controlling for age and mouthing behavior (OR = 1.9, 95% CI: [1.098, 3.350]). The likelihood of elevated blood Pb was more pronounced in anemic children younger than 18 months (OR = 3.1, 95% CI: [1.3, 7.4]).

Similarly, in a cross-sectional study of 340 children (aged 1–5 years) from Karachi, Pakistan, mildly-anemic and severely-anemic children (mean [SD] Hb levels: 8.9 [0.9] and 7.4 [0.5] g/dL, respectively) had lower Hb levels but higher blood Pb levels compared to non-anemic children (mean [SD] Hb: 12.1 [1.3] g/dL). Mean [SD] blood Pb levels in the mildly-anemic, severely-anemic, and non-anemic children were 14.9 [0.81], 21.4 [2.7], and 7.9 [1.7] µg/dL, respectively ($p < 0.01$) (Shah et al., 2010). Additionally, Hct, RBC count, and MCV were all decreased in anemic children versus non-anemic children. Although statistical analyses were not reported, the levels of Hb, Hct, RBC count, and MCV in anemic children all fell outside of the reported normal range for these parameters, whereas the reported values in non-anemic children did not. Blood Pb level was negatively correlated with Hb level in all groups, with the magnitude of negative correlation increasing with increasing severity of anemia: r = -0.315 (non-anemic children), -0.514 (mildly-anemic), and -0.685 (severely- anemic). In Fe-deficient anemic

children (n = 23) from Denizli, Turkey, mean (SD) serum Pb levels were statistically ($p < 0.05$) increased compared to healthy children (n = 179): 0.013 (0.004) versus 0.008 (0.001) µg/dL, respectively (Turgut et al., 2007). The Fe-deficient children were observed to have decreased Hb, MCV, RBC, and ferritin compared to controls, but increased RBC distribution width. In 140 children from southern Brazil aged 2–11 years, living within 25 km of a Pb smelter, blood Pb levels were not observed to differ between anemic and non-anemic children (mean [SD]: 10.36 [6.8] versus 9.73 [5.8] µg/dL, $p = 0.98$) (Rondo et al., 2006). However, blood Pb levels were significantly negatively correlated with Hb in anemic children ($r = -0.41$, $p = 0.01$); this relationship was not observed in non-anemic children ($r = 0.018$, $p = 0.84$).

In children aged 5–9 years (n = 189) without anemia living in Cartagena, Columbia, a smaller percentage (4.7%) of children had blood Pb >10 µg/dL (mean [SD]: 5.49 [0.23] µg/dL). The only hematological parameters that fell outside of their reference values were MCV and MCH, which were negatively correlated with blood Pb levels ($r = -0.159$ [$p = 0.029$] and -0.171 [$p = 0.019$], respectively) (Olivero-Verbel et al., 2007). RBC count, which was not observed to differ from reference values, was positively correlated with blood Pb level ($r = 0.208$, [$p = 0.004$]). In a group of 268 Lebanese children, children aged 11–23 months with blood Pb levels >10 µg/dL had increased likelihood of having Fe-dependent anemia and transferrin saturation (TF <12%) compared to age-matched children with blood Pb levels <10 µg/dL (OR = 4.59, 95% CI: [1.51, 13.92]) (Muwakkit et al., 2008). In children aged 24-35 months, higher blood Pb level was not associated with increased likelihood of either effect. Huo et al. (2007) found that children (less than 6 years of age) living near an area where electronic waste was recycled in China had significantly higher mean blood Pb levels than did children in the neighboring town with no waste recycling (15.3 versus 9.94 µg/dL). However, contrary to the findings above, no difference was detected in the mean Hb levels of the children in the two towns (12.76 g/dL in children from the waste recycling town versus 12.35 g/dL in children from the town with no recycling).

In adult, occupationally exposed populations, decreased erythrocyte numbers and Hb were observed in multiple, earlier cross-sectional studies investigating workers with blood Pb levels >40 µg/dL (Solliway et al., 1996; Horiguchi et al., 1991; Poulos et al., 1986). However, a larger, longitudinal study (Hsiao et al., 2001) observed that occupationally-exposed adults exhibited erythrocyte counts and Hct that were positively associated with blood Pb levels. Most of the recent occupationally-exposed groups represent populations highly exposed to Pb, with mean blood Pb levels ranging from 26–74 µg/dL. Although effects observed within these groups may not be generalizable to the general population as a whole, they are useful in demonstrating consistent effects on a number of hematological parameters, including Hb, MCV, MCH, MCHC, total RBCs,

and packed cell volume (PCV) (Khan et al., 2008; Patil et al., 2006a; Patil et al., 2006b; Karita et al., 2005).

A few recent cross-sectional occupational studies did investigate the effect of moderate occupational Pb exposure on hematological parameters. In gas station attendants in Sarajevo, Bosnia and Herzegovina, workers (mean [SD] duration of exposure: 12.1 [9.1] years) had significantly increased blood Pb levels (mean: 5.96 µg/dL) in 2008, compared to the same population that was previously examined in 2003 (mean: 4.07 µg/dL; mean [SD] duration of exposure: 10.4 [5.5]). Levels of MCH and MCHC were significantly decreased when assessed in 2008, compared to the 2003 measurements, although RBC numbers, Hb, Hct, and MCV were increased in 2008 compared to 2003. Positive correlations were observed in all subjects between blood Pb and RBC count, Hb, and MCH ($r = 0.241$, 0.201, and 0.213, respectively; $p < 0.05$). No control group was included in this study (Cabaravdic et al., 2010). Ukaejiofo et al. (2009) studied the hematological effects of Pb in 81 male subjects moderately exposed to Pb at three different manufacturing companies in Nigeria for durations between six months and 20 years. The exposed individuals had a mean blood Pb level of 7.00 µg/dL (± 0.07 µg/dL) compared to 3 µg/dL (± 0.19 µg/dL) in controls drawn from industries not involved in Pb handling (control group I) and 2 µg/dL (± 0.04 µg/dL) in controls drawn from the general population (control group II). It should be noted that the range of reported blood Pb levels in exposed workers ranged from 30 to 0 µg/dL. The limit of detection for blood Pb was reported as 0.05 µg/dL, meaning that there possibly is some bias towards zero in the reported mean blood Pb levels. Pb-exposed workers had significantly reduced Hb and PCV levels and increased percentage of reticulocytes compared to controls. Although the differences were statistically significant between the exposed and control subjects, the study authors stated that the levels in the exposed subjects were at the lower range of normal for Nigerians. The percent cell lysis did not significantly differ between controls and exposed workers; however, when workers and controls were stratified by age, there was a significant increase in cell lysis in workers under age 30 compared to similarly aged controls in group II ($p < 0.01$). Similarly, stratification of subjects by duration of exposure revealed that MCHC was decreased in exposed workers (6–60 months of exposure). Conterato et al. (2013) investigated hematological parameters in automotive painters exposed to Pb in Brazil. Exposed painters had a mean [SEM] blood Pb concentration of 5.4 [0.4] µg/dL compared to 1.5 [0.1] µg/dL in controls. The mean [SEM] duration of exposure to Pb in painters was 133.9 [14.5] months, whereas the controls were not occupationally exposed to Pb. Although Hct, Hb concentration, and the number of RBCs were significantly decreased in painters compared to controls, they were not correlated with blood Pb levels; however, these parameters were correlated with blood Cd^{2+} levels, which were also significantly elevated in painters compared to controls.

Taken together, the above occupational studies provide consistent evidence that high (mean blood Pb >26 µg/dL) occupational exposure to Pb reduces the number of RBCs in circulation. Additionally, the Ukaejiofo et al. (2009) study provides suggestive evidence that concurrent blood Pb levels below 10 µg/dL (7.0 µg/dL) may also be associated with decreased RBC survival. Although the decrease in RBCs observed in highly exposed worker populations may be explained by both decreased RBC survival and/or disruption of hematopoiesis, the observation of increased reticulocytes in Ukaejiofo et al. (2009) seems to represent compensation for decreased RBC survival due to Pb exposure.

In a non-occupational study, the associations between blood Pb levels, Ca^{2+}, Fe, and Hb were investigated in 55 pregnant Brazilian women (21.9% were 14–19 years old, 74.5% were 20–34 years olds, and 3.6% were ≥ 35 years old) (Zentner et al., 2008). The majority of women (across all age groups) had concurrent blood Pb levels <5 µg/dL (58.2%), although the mean blood Pb level was not reported; only 5.4% of women had blood Pb levels >10 µg/dL. The vast majority of the women (78.2%) were also observed to have adequate levels of Hb (≥ 11 g/dL). In a multiple linear regression model, blood Pb level was observed to be negatively associated with Hb (β = -0.359 per 1 µg/dL Pb), when controlling for age, BMI, income, energy intake, Ca^{2+} intake, vitamin C intake, and Fe intake.

The associations of blood Pb levels with hematological parameters observed in epidemiologic studies are clearly supported by a number of animal toxicology studies reporting blood Pb levels relevant to this ISA, i.e., <10 µg/dL. Hb concentrations in plasma (a marker of RBC hemolysis) was significantly increased in rats exposed to Pb acetate (1,000 ppm in drinking water for 9 months; blood Pb level: 7.1 µg/dL) compared to controls (Baranowska-Bosiacka et al., 2009). In a complementary in vitro experiment, a concentration-dependent increase in the amount of hemolysis was observed in human RBCs exposed to Pb at concentrations ranging from 0.1–100 µM for 5–30 minutes. Hemolysis was increased even at the lowest concentration tested (i.e., 0.1 µM). Pb-induced hemolysis in these experiments may be due to inhibition of RBC phosphoribosyltransferases (Section 4.7.2.5). In weanling rats (PND25 days, n = 10) whose dams were exposed to Pb acetate in drinking water (2.84 mg/mL, approximating mean [SD] daily exposures of 342.57 [28.11] and 744.47 [29.27] mg/kg [dam weight] during gestation and lactation, respectively), blood Pb level was significantly elevated compared to controls (mean [SE]: 69.8. [7.82] versus 0.54 [0.08] µg/dL). The only hematological parameter affected by Pb exposure was Hct, which was decreased in exposed rats (mean [SE]: 27.3 [0.5]%) versus controls (33.4 [0.3]%) (Molina et al., 2011). In rats treated with 25 mg Pb/kg by oral gavage for 4 weeks, the average plasma Pb concentration was 6.5 µg/dL (9.6-fold higher than controls), and statistically significant decreases in Hct, Hb, and RBCs were observed (Lee et al., 2005). Effects on

erythrocyte survival were similar in mice treated with Pb nitrate (50 mg/kg via gavage for 40 days): mean [SD] blood Pb levels were 1.72 [0.02] µg/dL versus 0.09 [0.011] µg/dL in control mice, and exposed mice had significantly reduced total RBC counts, total leukocyte counts, Hb, lymphocytes, and monocytes compared to controls ($p < 0.001$) (Sharma et al., 2010b).

A number of toxicological studies also reported similar hematological effects, but did not report final blood Pb concentrations. Rats exposed to Pb acetate (2 g/L in drinking water for 30 days) had significantly decreased RBCs, Hb, PCV, MCH, and MCHC compared to controls ($p < 0.05$) (Simsek et al., 2009), but not a disruption of hematopoiesis. Mice exposed to Pb acetate (1 g/L in drinking water for 90 days, but not those exposed for 15 or 45 days), had significantly decreased RBC counts and Hct compared to controls ($p<0.05$) (Marques et al., 2006). Spleen weights were observed to be increased relative to body weight in animals exposed to Pb for 45 days. Mice injected daily with Pb acetate (50 mg/kg subcutaneously) had significantly reduced Hb, MCV, MCH, and MCHC compared to controls injected with 5% dextrose (Wang et al., 2010h).

Some toxicological studies found no evidence of hematological effects in animals following exposure to Pb. Male rats exposed to Pb acetate in their drinking water for 4 weeks at concentrations ranging from 100–1,000 ppm had a concentration-dependent increase in blood Pb levels (range: 6.57–22.39 µg/dL) compared to controls (0.36 µg/dL), but there were no significant changes in any of the hematological parameters (complete blood cell count performed) measured at the end of treatment (Lee et al., 2006b). Slight, statistically nonsignificant increases in PS expression on RBC membranes were also observed. Similarly, exposure of male rats to 5,000 ppm Pb nitrate in drinking water (blood Pb not reported) for three weeks had no affect on any measured hematological parameter (Gautam and Flora, 2010). In vitro experiments with rat and human blood did not demonstrate a significant increase in hemolysis after 4 hours of treatment with Pb acetate at concentrations up to 10 µM.

Although Pb exposure has been consistently shown to shorten RBC life span and alter RBC mobility, as of the 2006 Pb AQCD, the mechanism of this was not well understood. While the mechanism is still not fully understood, there has been some indication for a role of free Ca^{2+}. Occupational studies investigating highly Pb-exposed worker populations (mean blood Pb >28 µg/dL) observed increased intracellular free Ca^{2+} levels ($[Ca^{2+}]_i$) in RBCs, and decreased RBC membrane $Ca^{2+/}Mg^{2+}$ATPase activity in workers compared with unexposed controls (Abam et al., 2008; Quintanar-Escorza et al., 2007). $[Ca^{2+}]_i$ levels were highly correlated with blood Pb levels even among unexposed control populations with mean blood Pb levels of approximately 10 µg/dL (9.9 ± 2 µg/dL) (Quintanar-Escorza et al., 2007). Changes in $[Ca^{2+}]_i$ were associated with increased

fragility of the RBCs and dramatic morphological alterations, including the increased presence of echinocytes (cells without normal biconcave shape) and crenocytes (speculated cells) in Pb-exposed workers.

Similar to the associations observed in Quintanar-Escorza et al. (2007), $[Ca^{2+}]_i$ increased in a concentration-dependent manner when RBCs from healthy human volunteers were exposed (in vitro) to 0.2 or 0.4 µM Pb nitrate for 24 or 120 hours (0.4 µM Pb nitrate roughly approximates 10 µg/dL Pb, although concentrations in exposure media are not directly comparable to blood Pb levels) (Quintanar-Escorza et al., 2010). The increase in $[Ca^{2+}]_i$ levels was observed to be related to increased Ca^{2+} influx and decreased efflux. As was observed among highly Pb-exposed workers, changes in $[Ca^{2+}]_i$ were associated with increased fragility of the RBCs and dramatic morphological alterations following exposure to 0.4 µM Pb. Similarly, Ciubar et al. (2007) found that RBC morphology was disrupted, with ≥ 50% RBCs having lost the typical discocytic morphology and displaying moderate to severe echinocytosis following exposure to Pb nitrate concentrations of 0.5 µM or higher for 24 hours. Exposure of RBCs to higher concentrations (concentrations not stated) of Pb nitrate resulted in cell shrinkage. In rats exposed to 200 ppm Pb acetate via drinking water for three months (mean [SD] blood Pb level: 40.63 [9.21] µg/dL), the cholesterol/phospholipid ratio of RBC membranes was increased, indicating that RBC membrane fluidity was decreased.

Khaĭrullina et al. (2008) observed that the surface profiles of RBC membrane shadows incubated with 0.5–10 µM Pb acetate for three hours were much smoother than were untreated RBC membranes when examined by atomic force microscopy. The authors postulated that the observed smoothing in Pb-treated RBC membranes may be due to clusterization of band 3 protein. Band 3 (anion exchanger 1 [AE1]), is a chloride/bicarbonate (Cl^-/HCO_3^-) exchanger and is the most abundant protein in RBC membranes. AE1 is integral in carbon dioxide (CO_2) transport and linkage of the cellular membrane to the underlying cytoskeleton (Akel et al., 2007; Su et al., 2007). The observed smoothing of the RBC membrane may due to Pb interfering with how the membrane attaches to the cytoskeletal structure of the RBC through perturbation of the normal activity of AE1.

Eryptosis

Eryptosis is the suicidal death of RBCs. It is characterized by cell shrinkage, membrane blebbing, and cell membrane phospholipid scrambling associated with PS exposure on the cell membrane that leads to cell destruction via macrophages (Föller et al., 2008; Lang et al., 2008). As previously reported in the 2006 Pb AQCD (U.S. EPA, 2006b), Kempe et al. (2005) found that exposing human RBCs to Pb at concentrations ranging

from 0.3 µM to 3 µM caused increased activation of K^+ channels that led to cell shrinkage and scramblase activation. The activation of scramblase increased the exposure to PS on the cell membrane, which causes an increase in the destruction of the RBCs by macrophages.

Consistent observations were made in recent studies that included in vitro and in vivo evidence. Shin et al. (2007) found that in vitro exposure of human RBCs to 1–5 µM Pb acetate increased PS expression in a time- and concentration-dependent manner. The maximum mean [SE] increase in expression of PS was 26.8% [3.15] (compared to deionized water), following exposure to 5 µM Pb for four hours. Scramblase activity was increased in Pb-exposed RBCs, and $[Ca^{2+}]_i$, which regulates scramblase activation, was also increased in exposed RBCs. Flippase, which translates PS exposure to inner membranes, is inhibited by high levels of $[Ca^{2+}]_i$ and was observed to exhibit reduced activity following Pb exposure. The inhibition of flippase is additionally influenced by the depletion of cellular adenosine triphosphate (ATP). ATP levels were decreased in a concentration-dependent manner following exposure to Pb. To corroborate these findings in vivo, Shin et al. (2007) treated male rats with Pb acetate (i.p. to 25, 50, or 100 mg/kg; blood Pb not reported). Expression of PS was observed to increase in a concentration-dependent manner at concentrations ≥ 50 mg/kg, confirming the in vitro results. No hemolysis or microvesicle formation was observed in the in vitro or in vivo experiments.

In a follow-up study, the same laboratory observed that in vitro exposure of human RBCs to much lower concentrations of Pb acetate (0.1, 0.25, and 0.5 µM) also induced PS expression. Most notably, exposure to 0.1 µM Pb for 24 hours increased PS expression on RBC membranes by approximately 20% (Jang et al., 2011). Accompanying the increased expression of PS (associated with Pb exposure) was the presence of abnormal echinocytic RBCs. Unlike the Shin et al. (2007) study described above, incubation of the RBCs with low concentrations of Pb (0.1 µM) induced the generation of microvesicles, which also expressed PS on their membranes in this (Jang et al., 2011) study. At 0.5 µM, Pb-exposed RBCs with externalized PS were observed to be targeted and engulfed by differentiated macrophages. Similar ex vivo effects were observed in rat erythrocytes four hours after oral exposure (0, 10 and 50 mg/kg) to Pb, although higher concentrations were generally required. PS expression on the rat erythrocytes was also observed. To corroborate these in vitro and ex vivo findings, rats were also exposed in vivo to 0, 50, 250, or 1,000 ppm Pb acetate in drinking water for 4 weeks. At 1,000 ppm, Hb and Hct were significantly decreased relative to control, and liver and spleen weights were increased. At the two highest doses, Fe accumulation was observed in the spleen, a clear sign of increased RBC clearance via phagocytosis.

Ciubar et al. (2007) also found that exposure to Pb nitrate (0.5–2 µM) resulted in an increase in PS exposure in RBCs and cell shrinkage, which the authors stated were indicators of cell apoptosis. As reported above, Khaĭrullina et al. (2008) observed Pb-induced RBC membrane smoothing that may be due to alterations in AE1 activity. Disruptions in AE1 activity may also result in enhanced PS exposure and premature cell death. Akel et al. (2007) observed that in $AE1^{-/-}$ knockout mice, Pb-induced PS exposure was much greater than that in wild type mice. Decreased RBCs and increased reticulocytes were also observed, an indication of high cell turnover.

4.7.2.3 Red Blood Cell Hematopoiesis

Erythropoietin (EPO) is a glycoprotein hormone excreted by the kidney to promote the development of RBCs in the bone marrow. As reported in the 2006 Pb AQCD, analyses of the cohort of children in Yugoslavia observed that EPO was increased in children aged 4.5 and 6.5 years of age living in a town near Pb sources (blood Pb levels >30 µg/dL) compared to children living in more distant town (blood Pb levels <10 µg/dL), when stratified by Hb concentrations (Graziano et al., 2004; Factor-Litvak et al., 1999; Factor-Litvak et al., 1998). These differences were not observed in children aged 9.5 or 12 years. With adjustment for Hb concentrations, blood Pb levels were observed to be significantly associated with EPO levels at ages 4.5 and 6.5 years when considering all children together. No significant association was observed at ages 9.5 and 12 years. Hb was not observed to differ at any age between towns, thus possibly indicating that hyperproduction of EPO is necessary to maintain Hb levels in young children living near Pb sources. The authors postulated that increases in EPO in younger children reflect bone marrow hyperactivity to counteract RBC destruction, whereas the lack of EPO elevation in older children may reflect a transitional period where increasing renal and bone marrow toxicity leads to decreases in EPO observed later in life, as observed in anemic, pregnant women (Graziano et al., 1991). Decreased EPO concentrations were also observed in association with Pb exposure in adults in two cross-sectional studies cited in the 2006 Pb AQCD (Osterode et al., 1999; Romeo et al., 1996).

Consistent with findings that EPO is negatively associated with blood Pb levels in adults, Sakata et al. (2007) observed that non-anemic tricycle taxi drivers (n=27) working in Kathmandu, Nepal (blood Pb level: 6.4 µg/dL) had significantly lower levels of EPO (12.7 versus 18.8 mU/mL) compared to non-driver controls (blood Pb level: 2.4 µg/dL). In taxi drivers, there was an inverse relationship between the level of serum erythropoietin and blood Pb level ($r = -0.68$, $p < 0.001$). Blood Pb level was not associated with any other hematological effects.

Recent toxicology studies of cytotoxicity and genotoxicity in RBC precursor cells support the observations that Pb exposure disrupts normal hematopoiesis. Cytotoxicity and genotoxicity in RBC precursor cells are strong indications of altered hematopoiesis in bone marrow. Celik et al. (2005) observed that treatment of female rats with Pb acetate (140, 250, or 500 mg/kg via gavage once per week for 10 weeks; blood Pb not reported) resulted in decreased numbers of polychromatic RBCs (PCE) and increased numbers of micronucleated PCEs, compared to controls ($p < 0.001$). Alghazal et al. (2008b) exposed male and female rats to 100 mg/L Pb acetate daily in drinking water for 125 days (blood Pb not reported) and observed increases in micronucleated PCEs in female rats ($p = 0.02$) but no significant reduction in the ratio of PCEs to normochromic RBCs (NCE). In male rats, an increase in micronucleated PCEs was observed ($p < 0.001$) along with a decrease in the PCE/NCE ratio ($p = 0.02$). While the results from Alghazal et al. (2008b) indicate that Pb is cytotoxic in male rats only, but is genotoxic in both sexes, results from Celik et al. (2005) indicate that Pb is cytotoxic in female rats as well. Mice exposed to Pb acetate (1 g/L in drinking water for 90 days; blood Pb not reported) had statistically significant increases in micronucleated PCEs; a small, but statistically nonsignificant decrease in the PCE/NCE ratio was also observed (Marques et al., 2006).

4.7.2.4 Membrane Proteins

While there have been few studies, evidence included in the 2006 Pb AQCD indicated there are effects of Pb on changes in RBC proteins. Huel et al. (2008) found that newborn hair and cord blood Pb levels (mean [SD]: 1.22 [1.41] µg/g and 3.54 [1.72] µg/dL respectively) were negatively associated with Ca^{2+}ATPase activity in plasma membranes of RBCs isolated from cord blood after controlling for gestational age and maternal Ca pump activity. However, newborn hair Pb levels were more strongly associated with cord Ca^{2+}-pump activity than were cord blood Pb ($p < 0.0001$ versus $p < 0.05$). Maternal blood Pb levels were not correlated with Ca^{2+}-pump activity in maternal or newborn cord blood. Pb-induced disruptions in Ca^{2+} homeostasis in RBCs can lead to cytotoxicity and necrosis, and these effects may be representative of cellular dysfunction in other organ systems.

In RBC membranes from Pb-exposed workers, Fukumoto et al. (1983) used polyacrylamide electrophoresis analysis and found increased levels of polypeptides in bands 2, 4, 6, and 7 and decreased levels of polypeptides in band 3. Apostoli et al. (1988) found changes in RBC membrane polypeptides, including a significant decrease in band 3, in occupationally exposed adults with blood Pb levels greater than 50 µg/dL. Apostoli et al. (1988) suggested that band 3 may represent an anion channel protein, whereas Fukumoto et al. (1983) suggested that the changes in the RBC membrane polypeptides

may cause changes in membrane permeability. Exposure to Pb acetate at concentrations above 0.1 µM for 60 minutes has also been found to increase the phosphorylation of proteins in human RBC membranes in vitro (Belloni-Olivi et al., 1996). Phosphorylation did not occur in cells depleted of protein kinase C (PKC), indicating a PKC-dependent mechanism.

4.7.2.5 Red Blood Cell Energy Metabolism Enzymes

RBCs use high energy purine nucleotides (i.e., ATP and guanine triphosphate [GTP]) to support basic metabolic functions. In mature RBCs, these nucleotides are synthesized via salvage reactions through either an adenine pathway, which requires adenine phosphoribosyltransferase (APRT), or an adenosine pathway, which requires adenosine kinase. The 2006 Pb AQCD (U.S. EPA, 2006b) reported that Pb significantly reduces the nucleotide pool (including NAD and NADP), as well as increases purine degradation products resulting in altered RBC energetics. Since the 2006 Pb AQCD was published, there have been few studies examining Pb effects on energy metabolism. Baranowska-Bosiacka et al. (2009) examined the effects of Pb on RBC APRT and hypoxanthine-guanine phosphoribosyltransferase (HPRT). In an in vitro experiment, APRT and HPRT were measured in lysate of human RBCs after exposure to Pb at a concentration range from 0.1 to 100 µM for 5–30 minutes. Complementary in vivo tests measured APRT and HPRT in RBC lysate from rats exposed to Pb acetate (1,000 ppm) in drinking water for 9 months. Both the in vivo and vitro studies found a significant decrease in both HPRT and APRT levels. The levels in human RBCs were significantly decreased in vitro after only 5 minutes of exposure to the 0.1 µM concentration, and the decrease was also concentration-dependent. However, the study authors considered the inhibition moderate (30–35%) even with the highest Pb levels used in vitro. Shin et al. (2007) found a concentration-dependent decrease in intracellular ATP in human RBCs in vitro with significant decreases, found even with the lowest concentration (i.e., 1 µM).

4.7.2.6 Other Enzymes

The 2006 Pb AQCD (U.S. EPA, 2006b) reported that K^+ permeability was increased by Pb exposure due to altered sensitivity of the membrane Ca^{2+}-binding site that caused selective efflux of K^+ ions from the RBC membrane. However, inhibition of the RBC Na^+/K^+ATPase is more sensitive to Pb exposure than is the inhibition of Ca^{2+}/Mg^{2+}ATPase. Few recent studies were found that examined the effects of Pb exposure on other enzymes. Ekinci et al. (2007) tested the effects of Pb exposure on two carbonic anhydrase isozymes (I and II) isolated from human RBCs. Carbonic anhydrases

are metalloproteins that use Zn to catalyze the equilibrium between CO_2 and bicarbonate in the cells of higher invertebrates. Although investigators found that Pb nitrate inhibited both carbonic anhydrase isozymes in a concentration-dependent manner, the concentrations used (i.e., 200–1,000 µM) were above those that would be physiologically relevant. Inhibition of isozyme I was noncompetitive, while the inhibition for isozyme II was uncompetitive. Bitto et al. (2006) examined the mechanisms of action of Pb-induced inhibition of P5N, an enzyme important in the pyrimidine salvage pathway that requires Mn for normal activity. Pb was observed to bind directly to the active site of the enzyme in a different position than the Mn, thus possibly resulting in improper protein folding and inhibition of activity.

4.7.2.7 Red Blood Cell Oxidative Stress

It has been suggested that the Pb-associated decreases in ALAD activity result in increased oxidative stress, owing to the buildup of ALA. ALA can act as an electron donor in the formation of reactive oxygen species (ROS) (Nemsadze et al., 2009; Ahamed and Siddiqui, 2007). Many epidemiologic and toxicological studies have found an association between the level of blood Pb and lipid peroxidation, antioxidant levels, or indicators of ROS production. The same limitations regarding cross-sectional studies listed in Section 4.7.2.2 (including uncertainty in directionality of effects and specific information regarding exposure) apply to the epidemiologic studies investigating RBC oxidative stress. Additionally, potential confounders and co-exposures were not considered in the majority of these studies. However, in studies where confounders were considered, they are explicitly delineated in the text.

Oxidative Stress, Lipid Peroxidation, and Antioxidant Enzymes

Malondialdehyde (MDA) is an end product of lipid peroxidation and is commonly measured as an indicator of oxidative stress. Evidence of lipid peroxidation has been observed in children moderately exposed to Pb. Ahamed et al.(2008; 2006, 2005) investigated the relationship between blood Pb levels and antioxidant enzyme levels and lipid peroxidation in children in India. Among children (N = 62) aged 4-12 years in Lucknow, India, children with mean blood Pb levels of 11.39 (SD: 1.39) µg/dL had increased measures of lipid peroxidation and decreased glutathione (GSH) levels compared to children with mean blood Pb levels of 3.93 (SD: 0.61) or 7.11 (SD: 1.25) µg/dL (Ahamed et al., 2005). Catalase activity was decreased in children with a mean 7.11 (SD: 1.25) µg/dL blood Pb level, compared to children with mean 3.93 (SD: 0.61) µg/dL blood Pb level. Additionally, blood Pb levels were found to be significantly

positively correlated with MDA and CAT, and negatively correlated with GSH. In a similar study, Ahamed et al. (2006) observed significantly higher levels of CAT and MDA in children with a mean 13.27 µg/dL blood Pb level compared to children with a mean 7.40 µg/dL blood Pb level, with other characteristics such as age, height, wieght, and BMI not differing between the two groups and thus, not considered as potential confounders. Examining all the study subjects together, investigators found a correlation between blood Pb level and blood MDA and RBC CAT levels, as well as an inverse relationship between ALAD activity and MDA and CAT levels. Among Indian children with neurological disorders, blood Pb levels were significantly increased compared to healthy control children (18.60 versus 10.37 µg/dL respectively) (Ahamed et al., 2008). Potential confounding characteristics such as age, sex, area of residence, and SES were not observed to be statistically different between the two groups, and therefore, were not included in statistical analyses. In addition, the following indicators of oxidative stress were elevated among case children: increased blood MDA, RBC SOD and CAT levels, and decreased blood GSH levels. GPx levels were similar between the two groups. Typical indicators of Pb exposure (active/nonactive ALAD ratio) were found to be correlated with lipid peroxidation and oxidative stress. Children aged 3–6 years old living near a steel refinery in China with blood Pb levels ≥ 10 µg/dL also had a significant increase in plasma MDA compared to children with blood Pb levels <10 µg/dL. However, levels of RBC SOD, GSH, and GPx were not different from those in controls (Jin et al., 2006).

Evidence of lipid peroxidation was also observed in occupational cohorts moderately exposed to Pb. In auto repair apprentices in Turkey (mean [SD]: 16.8 [1.2] years of age, 3.8 [1.8] years duration of exposure) with mean blood Pb levels of 7.9 µg/dL (Ergurhan-Ilhan et al., 2008), increases in glutathione peroxidase (GPx) and MDA, as well as decreases in α-tocopherol and β-carotene were observed compared with controls (mean [SD] age: 16.3 [1.0] years, mean blood Pb level: 2.6 µg/dL). Decreases were observed in SOD and CAT, but the results did not attain statistical significance. Statistically significant alterations in measures of oxidative stress were also observed in other occupationally exposed populations. SOD, GSH, and CAT were decreased; while oxidized GSH (i.e., GSSG) and thiobarbituric acid reactive species (TBARS, expressed in terms of MDA) were increased in painters in India (mean [SD] duration of exposure: 126.08 [49.53 months], mean blood Pb level: 21.92 µg/dL, compared to 3.06 µg/dL in controls) (Mohammad et al., 2008). Glutathione-S-transferase, GPx, and SOD were positively correlated with blood Pb levels (mean: 5.4 µg/dL, r = 0.34, 0.38, and 0.32, respectively; $p < 0.05$) in automotive painters in Brazil (Conterato et al., 2013).

Numerous cross-sectional, occupational studies have also demonstrated increased lipid peroxidation in highly-exposed worker populations (blood Pb levels ranging from 29.0 to 74.4 µg/dL) (Kasperczyk et al., 2009; Khan et al., 2008; Quintanar-Escorza et al., 2007; Patil et al., 2006a; Patil et al., 2006b). There was a correlation between MDA levels and blood Pb levels, even in the unexposed workers who had lower (i.e., <12 µg/dL) blood Pb levels, although the magnitude of correlation in exposed workers was greater (Quintanar-Escorza et al., 2007). Increases in C-reactive protein and decreases in RBC SOD, CAT, and plasma ceruloplasmin were also observed in these workers, further indicating increased RBC oxidative stress due to higher Pb exposure.

Oral administration of Pb (25 mg/kg) to rats once a week (i.e., bolus gavage) for 4 weeks, which produced a plasma Pb level of about 6.5 µg/dL, caused a significant increase in RBC MDA levels (Lee et al., 2005). Other indications of Pb-induced oxidative stress included significant increases in RBC SOD and CAT levels accompanied by significant decreases in GSH and GPx. Exposure of rats to Pb acetate (750 mg/kg in drinking water for 11 weeks) resulted in decreased concentrations of plasma vitamin C, vitamin E, nonprotein thiol, and RBC-GSH, with simultaneous increased activity of SOD and GPx (Kharoubi et al., 2008b). CAT activity was also slightly elevated in RBCs from the Pb-exposed rats, but the increase failed to reach statistical significance. Exposure of male rats to 5,000 ppm Pb nitrate in drinking water (blood Pb not reported) for three weeks decreased GSH levels compared to that in controls (mean [SE]: 1.91 [0.02] versus 2.44 [0.09] mg/mL, respectively) (Gautam and Flora, 2010). SOD activity was significantly decreased in rats injected with Pb acetate (15 mg/kg, i.p. for seven days, but not rats injected with 5 mg/kg) (Berrahal et al., 2007). GPx activity and MDA concentrations were slightly elevated in the two exposed groups, but differences with the control (15 mg Na acetate/kg) group failed to reach statistical significance. Effects on indices of oxidative stress were also observed in in vitro studies: increased MDA and decreased SOD and CAT in RBCs exposed to 2 µM Pb (Ciubar et al., 2007), decreased glutathione reductase (GR) activity in human RBCs incubated with 5–18 µM Pb (Coban et al., 2007), and decreased GSH and increased GSSG and lipid peroxidation in RBCs from healthy volunteers (with no history of Pb exposure) incubated with 0.4 µM Pb for 24–120 hours (Quintanar-Escorza et al., 2010).

Antioxidant Defense

In addition to the studies listed above that examined lipid peroxidation and oxidative stress, there have been toxicological studies that indicate that the use of antioxidants and free radical scavengers is protective against Pb-induced RBC oxidative stress. Rats treated with 500 ppm Pb acetate in drinking water for 15 or 30 days had a significant increase in free RBC protoporphyrin and TBARS levels that was related to length of

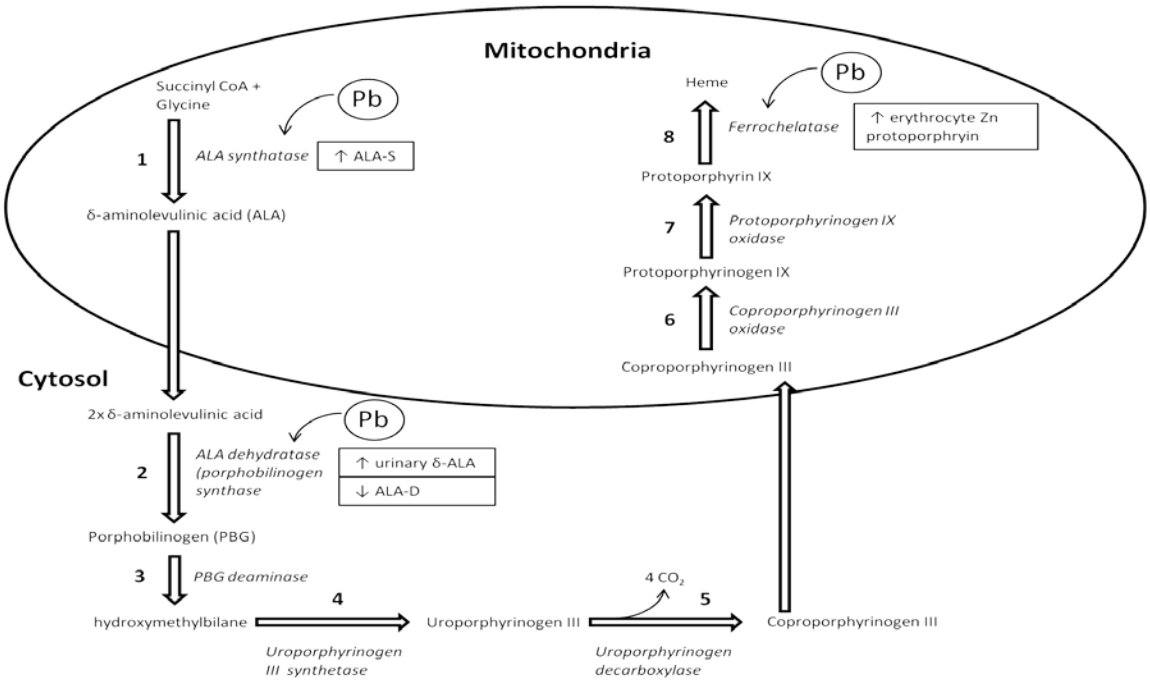
exposure and blood Pb levels (Rendón-Ramirez et al., 2007). Vitamin E administration after exposure to Pb significantly reduced the rat RBC TBARS levels and increased ALAD activity, compared to exposure to Pb alone. Co-exposure to vitamin E and Pb simultaneously and exposure to vitamin E before Pb exposure also prevented Pb-induced oxidative stress. In vitro studies by Casado et al. (2007) found that Pb-induced hemolysis using blood from non-occupationally exposed volunteers indicated that RBC membrane damage was mediated via oxidative stress. The in vitro studies demonstrated a concentration- and time-dependent formation in lipid peroxide that was inhibited with a number of antioxidants, including desferrioxamine (Fe chelator), trolox (chain breaking antioxidant), and mannitol and Na formate (OH scavengers). Results suggested the role of singlet oxygen in Pb-mediated membrane damage and hemolysis of exposed RBCs. In rats exposed to 2,000 ppm Pb in drinking water for 5 weeks, MDA levels were significantly increased, whereas vitamin E concentrations were significantly decreased (Caylak et al., 2008). In the case of MDA, co-exposure to Pb and a number of sulfur-containing antioxidants (e.g., L-methionine, N-acetylcysteine, and L-homocysteine) reduced concentrations to a level not significantly different from that in controls, but were significantly smaller than concentrations observed with Pb alone. Exposure to L-methionine and N-acetylcysteine also reduced Pb-induced depletion of vitamin E.

4.7.2.8 Summary of Effects on RBC Survival and Function

In summary, Pb exposure has been shown to affect multiple hematological outcomes that are related to RBC survival and function, as demonstrated in both cross-sectional epidemiologic studies and toxicological studies. Pb exposure has been shown to decrease RBC survival, either through direct effects on RBC membranes leading to increased fragility, or through the induction of eryptosis and eventual phagocytosis by macrophages. Limited evidence that Pb can negatively affect hematopoiesis is also available. Consistent evidence also exists demonstrating that Pb exposure increases oxidative stress in exposed adults and children, as well as in laboratory animals. The epidemiologic studies demonstrating these effects are cross-sectional in design, therefore there is some uncertainty regarding the direction of effects and the magnitude, timing, frequency, and duration of Pb exposure that contributed to the observed observations. Also, the majority of epidemiologic studies did not account for potential confounding, although the effects observed in these studies are consistent with effects from studies that did consider potential confounding. The coherence with effects observed in animal toxicology studies supports the conclusion that Pb exposure affects both the survival and function of RBCs.

4.7.3 Red Blood Cell Heme Metabolism

Pb exposure has been found to inhibit several enzymes involved in heme synthesis, namely ALAD (a cytoplasmic enzyme catalyzing the second, rate-limiting, step of the heme biosynthesis pathway), coproporphyrinogen oxidase (catalyses the sixth step in heme biosynthesis converting coproporphyrinogen III into protoporphyrinogen IX), and ferrochelatase (catalyses the terminal [eighth] step in heme synthesis converting protoporphyrin IX into heme) (Figure 4-35). The observations of decreased Hb (measured as total Hb, MCH, or MCHC) in occupationally-exposed adults (Ukaejiofo et al., 2009; Khan et al., 2008; Patil et al., 2006b; Karita et al., 2005) and Pb-exposed experimental animal models (Sharma et al., 2010b; Baranowska-Bosiacka et al., 2009; Simsek et al., 2009; Marques et al., 2006; Lee et al., 2005) and associations with blood Pb levels in children (Queirolo et al., 2010; Shah et al., 2010; Olivero-Verbel et al., 2007; Riddell et al., 2007) are supporting lines of evidence for decreased heme synthesis due to Pb exposure.



Note: Steps in the pathway potentially affected by Pb are indicated with curved arrows pointing to the affected enzyme, and the directions of effects are represented by ↑ and ↓ arrows.

Figure 4-35 Schematic representation of the enzymatic steps involved in the heme synthetic pathway.

4.7.3.1 Red Blood Cell 5-Aminolevulinic Acid Dehydratase

Decreases in RBC 5-aminolevulinic acid dehydratase (ALAD) levels are strongly associated with Pb exposure in humans; to such an extent that RBC ALAD activity is used as a biomarker to assess Pb toxicity. Several epidemiologic studies published since the 2006 Pb AQCD evaluated the relationship between Pb exposure, blood Pb levels and ALAD activity in adults and children (see below). These studies were cross-sectional in nature. This limits their utility in assessing the direction of effects and the magnitude, timing, frequency, and duration of Pb exposure necessary to contribute to the observed associations. In studies that considered potential confounders, those confounding variables are listed in the text. However, potential confounding was not accounted for in the majority of these studies.

Wang et al. (2010g) found that, after controlling for sex, age, alcohol consumption and smoking (adults only), there was a blood Pb concentration-dependent decrease in ALAD activity in both children (4–13 years old) and adults (16–77 years old) (mean blood Pb levels: 7.1 and 6.4 µg/dL, respectively) in rural southwest China. Further, Wang et al. (2010g) observed that the relationship between blood Pb level and ALAD activity was nonlinear and exponential, with larger decreases in ALAD activity occurring with blood Pb levels >10 µg/dL. No correlation was observed between urinary ALA levels and blood Pb levels. Ahamed et al. (2006) studied male urban adolescents in India. The 39 adolescents were separated into two groups according to their blood Pb levels (Group 1: <10 µg/dL [mean 7.4 µg/dL], Group 2: >10 µg/dL [mean 13.27 µg/dL]). Although Groups 1 and 2 were similar in age (mean [SD]: 16.59 [0.91] versus 16.76 [0.90] years, respectively), height, weight, and BMI (therefore not considered potential confounders), Group 2 (with the higher blood Pb levels) had lower ALAD activity than did Group 1 ($p < 0.001$). When all 39 adolescents were examined together, an inverse relationship was found between blood Pb and ALAD activity. Similar decreases in ALAD activity were observed in other populations of children from India (aged 4–12 and 1–7 years) with elevated blood Pb levels (mean [SD]: 11.39 [1.39] and 21.86 [7.58] µg/dL respectively) compared to the two age ranges of the children with lower blood Pb levels (mean [SD]: 3.93 [0.61] and 6.89 [2.44] µg/dL respectively) (Ahamed et al., 2007; Ahamed et al., 2005). While Ahamed et al. (2005) did not address potential confounding, Ahamed et al. (2007) observed decreases in ALAD activity after controlling for age, sex, and area of residence. Decreases in ALAD activity were also observed in children 3–6 years of age with Pb blood levels >10 µg/dL, compared to children <10 µg/dL (mean blood Pb concentration for groups not reported) in northeastern China (Jin et al., 2006).

As was seen with epidemiologic studies investigating Pb-associated deficits in hematological parameters, most occupational studies investigating ALAD levels may not

be generalizable to the population as a whole; however, they are useful in demonstrating consistent and negative effects of Pb exposure on the activity of this enzyme (Quintanar-Escorza et al., 2007; Patil et al., 2006a; Patil et al., 2006b; Ademuyiwa et al., 2005b). Occupationally-exposed adults (mean blood Pb levels: 47.4 µg/dL) had levels of inhibition of ALAD that were as great as 90% relative to control (blood Pb levels: 9.9 µg/dL)(Quintanar-Escorza et al., 2007). There were few studies that investigated Pb-associated decrements in ALAD levels among moderately-exposed workers. Painters in India with a mean blood Pb level of 21.92 µg/dL (mean [SD] duration of exposure: 126.08 [49.53] months) had lower ALAD levels ($p < 0.01$) compared to controls whose mean blood Pb level was 3.06 µg/dL (Mohammad et al., 2008). Stoleski et al. (2008) observed that workers in a Pb smelter in Macedonia (mean [SD]: 16.4 [8.5] µg/dL blood Pb; 18.8 [7.5] years employment) had lower ALAD activity ($p < 0.001$) and higher ALA levels ($p < 0.0005$) compared to workers with no history of exposure to Pb (mean [SD] blood Pb: 7.0 [5.4] µg/dL). In automotive painters exposed to Pb in Brazil (mean [SD]: 5.4 [0.4] µg/dL blood Pb level; 133.9 [14.5] months duration of exposure), the ALAD reactivation index was increased over that in controls, although ALAD activity did not differ between groups (Conterato et al., 2013). However, ALAD activity was negatively correlated with blood Pb levels ($r = -0.59$, $p < 0.05$) but not blood Cd levels, whereas ALAD reactivation index was positively correlated with blood levels of both metals (Pb: $r = 0.84$, $p < 0.05$; Cd: $r = 0.27$, $p < 0.05$). In a benchmark dose (BMD)-based analysis (benchmark response = 5% using the hybrid approach and a 5% adversity cut-off value), Murata et al. (2009) calculated the BMD and 95% lower confidence limit of the BMD (BMDL) for decreased ALAD activity in RBCs of exposed Pb workers. The calculated BMD (2.7 µg/dL) and BMDL (2.3 µg/dL) values for ALAD, respectively, were substantially lower than the BMDs (28.7–44.2 µg/dL) and BMDLs (19.4–29.6 µg/dL) for decreased Hb, Hct, and RBC count in similarly exposed workers, indicating decreases in ALAD activity can occur at blood Pb levels that do not decrease RBC survival.

Decreased ALAD activity in response to Pb exposure has also been observed in toxicological studies. Rats administered 500 ppm Pb acetate in drinking water for 15 or 30 days had decreased blood ALAD activity, which was related to duration of exposure and blood Pb levels (Rendón-Ramirez et al., 2007). Oral administration of Pb (25 mg/kg) to rats once a week for 4 weeks achieved a blood Pb level of 6.5 µg/dL, which was associated with statistically significant decreases (approximately 50% lower than control levels) in RBC ALAD activity (Lee et al., 2005). Exposure of male Wistar rats to 5,000 ppm Pb acetate via drinking water for three weeks significantly decreased ALAD activity by 72% (mean [SD]: 7.35 [0.35] versus controls: 26.14 [2.19] nmol/min/mL RBCs [nanomoles of porphobilinogen (PBG) formed per minute, per 1 mL blood]) (Gautam and Flora, 2010).

4.7.3.2 Other Heme Metabolism Enzymes

The 2006 Pb AQCD (U.S. EPA, 2006b) indicated that Pb affects RBC PBG synthase (Simons, 1995; Farant and Wigfield, 1990, 1987), PBG deaminase (Tomokuni and Ichiba, 1990), and TF endocytosis and Fe transport across membranes (Qian and Morgan, 1990), all of which are directly or indirectly involved in heme synthesis. Although there are no recent studies that examine the effect Pb has on the activities of other heme metabolism enzymes, a number of studies investigated associations of blood Pb level with concentrations of various intermediate products in the heme biosynthetic pathway.

Pb intoxication has been shown to inhibit the function of ferrochelatase, the enzyme that catalyzes the last (eighth) step in the heme biosynthetic pathway. Under normal conditions, ferrochelatase incorporates ferrous iron (Fe^{2+}) into protoporphyrin IX, converting it into a heme molecule (Figure 4-35). However, Pb has been shown to inhibit this insertion of Fe^{2+} into the protoporphyrin ring and instead, Zn is inserted into the ring creating ZPP. A number of recent studies have shown that blood Pb level is significantly associated with increased RBC ZPP levels in adults occupationally exposed to high levels of Pb (blood Pb levels: 27–54 µg/dL) (Patil et al., 2006b; Ademuyiwa et al., 2005b), workers exposed to moderate levels of Pb (blood Pb level = 21.92 µg/dL) (Mohammad et al., 2008), children aged 1–21 years (blood Pb levels: 18–23 µg/dL) (Counter et al., 2009, 2008; Counter et al., 2007), and animals exposed to 500 ppm Pb via drinking water for 15 or 30 days (blood Pb levels: 24.7 or 31.8, respectively) (Rendón-Ramirez et al., 2007). Interestingly, Wang et al. (2010g) found that in children and adults living in a rural area of Southwest China, ZPP levels were negatively correlated with blood Pb at blood Pb levels <10 µg/dL and were only positively correlated with blood Pb at higher blood Pb concentrations (i.e., >10 µg/dL). The authors suggested that this may be representative of ALAD activities at low blood Pb levels, which contributes to lower ZPP levels. Scinicariello et al. (2007) performed a meta-analysis and observed that Pb-exposed individuals who carried the ALAD2 allele had slightly lower concentrations of blood ZPP levels compared to carriers of the ALAD1 allele (overall pooled standardized mean estimate: -0.09 [ZPP value for ALAD 1-2/2-2 carriers vs ALAD1-1 carriers]; 95% CI: -0.22, 0.03, p = 0.13).

4.7.3.3 Hematological Effects

In summary, Pb exposure has been shown in both cross-sectional epidemiologic studies and toxicological studies to alter heme synthesis. Pb exposure has been shown to inhibit the activities of two major enzymes in the heme biosynthetic pathway: ALAD and ferrochelatase. Evidence for the inhibition of ALAD comes from direct measurements of

its activity in exposed human populations, whereas evidence for inhibition of ferrochelatase comes from the observation of increased ZPP levels in association with occupational exposure or blood Pb levels. Animal toxicology and ecotoxicology studies provide evidence of coherent effects in animals. The epidemiologic studies demonstrating these effects are cross-sectional in design, therefore there is some uncertainty regarding the direction of effects and the magnitude, timing, frequency, and duration of Pb exposure that contributed to the observed observations. Also, the majority of epidemiologic studies did not account for potential confounding, although the effects observed in these studies are consistent with effects from studies that did account for confounding. The coherency of effects observed in animal toxicology and ecotoxicology studies support the conclusion that Pb exposure alters the synthesis of heme in RBCs.

4.7.4 Summary and Causal Determination

Recent toxicological and epidemiologic evidence substantiates evidence presented in the 2006 Pb AQCD that exposure to Pb affects hematological endpoints, and supports a causal relationship between Pb exposure and decreased RBC survival and function and altered heme synthesis. Outcomes related to decreased RBC survival and function included alterations in multiple hematological parameters (e.g., Hb, Hct, PCV, MCV, MCH), oxidative stress (altered antioxidant enzyme activities [SOD, CAT, GPx], decreased cellular GSH, and increased lipid peroxidation), increased cytotoxicity in RBC precursor cells, and mode of action endpoints such as decreased intracellular calcium concentrations, decreased ATPase activity, and increased phosphatidylserine expression. Outcomes related to altered heme synthesis included decreased activities of ALAD and ferrochelatase, and decreased levels of Hb. The sections that follow describe the evaluation of evidence for decreased red blood cell (RBC) survival and function and heme synthesis, with respect to causal relationships with Pb exposure using the framework described in Table II of the Preamble. The application of the key evidence to the causal framework is summarized in Table 4-35.

4.7.4.1 Evidence for Decreased RBC Survival and Function

The 2006 Pb AQCD (U.S. EPA, 2006b) reported that Pb exposure is associated with multiple measures of decreased RBC survival and function. Epidemiologic evidence included the observation of a 10% probability of anemia with blood Pb levels of approximately 20 µg/dL at age 1 year, and perturbed hematopoiesis in children and adults at blood Pb levels between 10-40 µg/dL. Oxidative stress was also identified by the 2006 Pb AQCD as a potential mode of action for Pb-induced effects in RBCs. A causal

relationship between Pb exposure and decreased RBC survival and function is strongly supported by the available, recent toxicological and epidemiologic data. Among the strongest evidence for Pb-induced decreases in RBC survival and function is the consistent observation of alterations in hematological parameters (e.g., Hb, Hct, PCV, MCV, MCH), oxidative stress (altered antioxidant enzyme activities [SOD, CAT, GPx], decreased cellular GSH, and increased lipid peroxidation), and increased cytotoxicity in RBC precursor cells in rodents exposed to various forms of Pb via drinking water (Jang et al., 2011; Molina et al., 2011; Gautam and Flora, 2010; Baranowska-Bosiacka et al., 2009; Simsek et al., 2009; Alghazal et al., 2008b; Kharoubi et al., 2008b; Marques et al., 2006). Some of these effects have been observed in toxicological studies reporting blood Pb levels of 2-7 µg/dL, and therefore occur at blood Pb levels that are relevant to this ISA. These effects at relevant blood Pb levels were found primarily in adult animals with Pb exposure durations of 4 weeks to 9 months. Although not as representative of potential human exposure pathways as exposure via drinking water, numerous toxicological studies utilizing oral gavage have also observed effects on hematological parameters, oxidative stress, and hematopoiesis (Sharma et al., 2010b; Celik et al., 2005; Lee et al., 2005). The animal toxicological evidence for decreased RBC survival and function is particularly important to the weight of evidence as it establishes clear temporality of exposure to Pb and induction of effects on red blood cells.

Associations between increased blood Pb levels and decreased RBC survival and function, are also evident in diverse populations of human adults and children. Cross-sectional studies in children measuring concurrent blood Pb levels are consistent regarding effects on hematological parameters (Queirolo et al., 2010; Shah et al., 2010; Ahamed et al., 2007; Huo et al., 2007; Olivero-Verbel et al., 2007; Riddell et al., 2007; Turgut et al., 2007; Ahamed et al., 2006; Jin et al., 2006; Rondo et al., 2006). Associations between altered indices of RBC oxidative stress and blood Pb levels were also seen in adolescents and children (Ahamed et al., 2008; Ahamed et al., 2006; Jin et al., 2006). The blood Pb levels observed in cross-sectional studies of children tended to be lower than those observed in adult populations (see below), with the majority of studies in children (ages 5 months to 5 years old) reporting mean concurrent blood Pb levels <15 µg/dL (range: 6.9–21.86 µg/dL). The difference in blood Pb levels may reflect the comparatively shorter duration and lower magnitude of Pb exposure experienced by children compared to adults.

For adult populations, the largest body of evidence consists of Pb-exposed workers in which measures of RBC survival (e.g., Hb, Hct, PCV, MCV, MCH) are altered when compared with unexposed control populations in cross-sectional studies (Conterato et al., 2013; Cabaravdic et al., 2010; Ukaejiofo et al., 2009; Khan et al., 2008; Patil et al., 2006a; Patil et al., 2006b; Karita et al., 2005). Only one non-occupational study was

found investigating the association of Pb with hematological parameters; in pregnant women, concurrent blood Pb levels were found to be negatively correlated with Hb concentrations. Cross-sectional studies have also observed consistent increases in lipid peroxidation in occupationally-exposed adult populations (Ergurhan-Ilhan et al., 2008; Khan et al., 2008; Mohammad et al., 2008; Quintanar-Escorza et al., 2007; Patil et al., 2006a; Patil et al., 2006b), and have observed changes in oxidative stress parameters, including lowered activities of antioxidant enzymes such as SOD, GR, and CAT. Recent evidence of disrupted hematopoiesis, including the observation of decreased serum EPO in occupationally-exposed adults with a mean blood Pb level of 6.4 µg/dL (Sakata et al., 2007), was consistent with previous findings of decreased EPO in exposed adults reported in the 2006 Pb AQCD. Although the mean blood Pb level in most occupationally exposed populations was >20 µg/dL, multiple studies observed negative effects in occupationally-exposed populations with mean blood Pb levels <10 µg/dL, including significant correlations PCV (7 µg/dL), significant correlations between RBC distribution width and MCHC (5.4 µg/dL), and decreased EPO (6.4 µg/dL). Any differences in the effects on specific hematological and oxidative stress parameters between adult populations and children may reflect differences in exposure durations or patterns of exposure, although there is greater uncertainty regarding the timing and duration of exposure associated with these effects in adults.

The evidence for Pb-associated decrements in RBC function and survival in adults and children comes from cross-sectional studies measuring concurrent blood Pb levels, and thus, the temporality of effects and the timing and duration of exposure associated with altered RBC survival and function in RBCs is unclear. This uncertainty is greatest in adults and older children as concurrent blood Pb levels also reflect higher past Pb exposures. Additional limitations of the epidemiologic database include the general lack of controlling for potential confounders or other possible co-exposures to contaminants that can affect the hematological system. Although most studies did not control for potential confounders, a few studies investigating effects in children did adjust for potential confounders such as age, sex, area of residence, breastfeeding, mouthing behavior, family structure, anemia, dairy product consumption, maternal age, education, employment, marital status, and SES-related variables; and still observed negative effects on RBC survival and function. However, no studies controlled for nutritional status, including Fe intake. Further, while the epidemiologic database may be limited for the above reasons, the findings in these studies demonstrated coherence with findings from multiple toxicological studies that either reported blood Pb levels that are relevant to this ISA, i.e., <10 µg/dL (drinking water and gavage studies) or utilized a relevant route of exposure (drinking water), and reported clear evidence for decreased RBC survival and function.

The causal relationship between Pb exposure and decreased RBC survival and function is further supported by epidemiologic and toxicological evidence characterizing mode of action and biological plausibility. Pb was shown to reduce Ca^{2+}ATPase and Ca^{2+}/Mg^{2+}ATPase activities in RBC membranes, which leads to an increase in RBC $[Ca^{2+}]_i$, increased membrane fragility, and abnormal morphological changes in studies of occupationally exposed adults (Quintanar-Escorza et al., 2007) and in in vitro studies (Quintanar-Escorza et al., 2010; Ciubar et al., 2007). Heul et al. (2008) observed a reduction in plasma membrane Ca^{2+}ATPase pump activity in newborn children's RBC membranes in association with cord blood Pb level in a group with a mean blood Pb of 3.54 µg/dL. Pb exposure has also been observed to increase PS expression on RBC membranes, leading to cell shrinkage, erythropoiesis, and destruction of the RBCs by macrophages (Jang et al., 2011; Ciubar et al., 2007; Shin et al., 2007).

Experimental animal studies demonstrate that Pb exposures via drinking water and gavage, resulting in blood Pb levels relevant to this ISA, alter several hematological parameters, increase measures of oxidative stress, and increase cytotoxicity in RBC precursor cells. These effects were found primarily in adult animals with Pb exposure durations of 4 weeks to 9 months. Support for these findings is provided by biologically plausible modes of action, including decreased intracellular calcium concentrations, decreased ATPase activity, and increased phosphatidylserine expression. Epidemiologic studies demonstrate evidence in both adults and children that concurrent blood Pb levels are associated with altered hematological endpoints and increased measures of oxidative stress, and altered hematopoiesis. However, the majority of these studies are limited by the lack of rigorous methodology and consideration for potential confounding. Although there are limitations in the epidemiologic evidence, some studies in children did control for or considered potential confounding and effects in adults and children are coherent with effects observed in exposed animals. Because epidemiologic evidence is limited to associations with concurrent blood Pb levels, there is uncertainty regarding the timing, duration, magnitude, and frequency of Pb exposure associated with decreased RBC survival and function. Collectively, the strong evidence from toxicological studies that is supported by findings from mode of action and epidemiologic studies is sufficient to conclude that there is a causal relationship between Pb exposures and decreased RBC survival and function.

4.7.4.2 Evidence for Altered Heme Synthesis

The 2006 Pb AQCD (U.S. EPA, 2006b) reported that Pb exposure affects heme synthesis in humans and animals through the inhibition of multiple key enzymes in the heme biosynthetic pathway, including ALAD and ferrochelatase. A causal relationship between Pb exposure and altered heme synthesis is strongly supported by the available toxicological, ecotoxicological, and epidemiologic data (Table 4-35). The greatest weight of evidence for Pb-induced alterations in heme synthesis lies primarily in the toxicological and ecotoxicological literature. A small, but coherent, body of recent toxicological evidence demonstrates decreased ALAD activity (Gautam and Flora, 2010; Lee et al., 2005) and ferrochelatase (Rendón-Ramirez et al., 2007) in adult rats exposed to Pb via drinking water and oral gavage for 3-4 weeks. Lee et al. (2005) observed effects on ALAD activity in Wistar rats at mean blood Pb levels of 6.5 µg/dL after Pb administration by oral gavage once per week for 4 weeks. Evidence from previous studies cited in the 2006 Pb AQCD consistently observed Pb-induced ALAD inhibition in multiple species, including birds, primates, and humans, further supporting the causal association between Pb exposure and altered heme synthesis.

Similar to the earlier and more recent toxicological studies that demonstrate an association between Pb exposure and hematological effects in humans and laboratory animals, the ecological literature has consistently reported on hematological responses in Pb-exposed aquatic and terrestrial invertebrates and vertebrates (Sections 6.3.12.5, 6.4.12.5, and 6.4.21.5). The most consistently observed effect in metal impacted environments is decreased RBC ALAD activity. This effect has been observed across a wide range of taxa, including bivalves, fish, amphibians, birds, and mammals. More limited evidence exists regarding deleterious effects of Pb exposure on serum enzyme levels and white blood cell counts in birds and mammals.

Consistent associations between increased Pb blood levels and decreased activity of multiple enzymes involved in the heme synthetic pathway have also been observed in diverse populations of adults and children. The strongest evidence for altered heme synthesis in adults and children come from cross-sectional epidemiologic studies measuring concurrent blood Pb and reporting associations with decreases in RBC ALAD levels and activity (Conterato et al., 2013; Wang et al., 2010g; Mohammad et al., 2008; Ahamed et al., 2007; Quintanar-Escorza et al., 2007; Ahamed et al., 2006; Patil et al., 2006a; Patil et al., 2006b; Ademuyiwa et al., 2005b; Ahamed et al., 2005). In addition to ALAD inhibition, recent studies have also shown that blood Pb levels or Pb exposure inhibits the activity of ferrochelatase, leading to increased RBC ZPP levels in children and occupationally-exposed adults (Counter et al., 2009, 2008; Mohammad et al., 2008; Counter et al., 2007; Patil et al., 2006b; Ademuyiwa et al., 2005b). Although the mean

blood Pb levels in most of the studies investigating these effects in adults and children were >20 µg/dL, two studies did observe negative effects in populations with mean blood Pb levels <10 µg/dL: increased ALAD reactivation index in exposed painters (5.4 µg/dL), and statistically significant, positive associations between ALAD and blood Pb level in children and the elderly (7.1 and 6.4 µg/dL, respectively).

The cross-sectional nature of the above epidemiologic studies in adults and children, and the measurement of concurrent blood Pb, introduces some uncertainty regarding the temporality of effects and the timing and duration of exposure associations with altered heme synthesis. Although most studies did not control for potential confounders, a few studies investigating effects in children, and one study investigating effects in adults, did adjust for confounders such as age, sex, urban/rural residence, height, weight, BMI, smoking status, and alcohol use, and still observed negative effects on heme synthesis. However, no studies controlled for nutritional status, includingFe intake. Further, while the epidemiologic database may be limited for the above reasons, the findings in these studies demonstrated coherence with findings from multiple toxicological and ecotoxicological studies.

The causal relationship between Pb exposure and altered heme synthesis is further supported by cross-sectional studies observing decreased Hb (measured as total Hb, MCH, or MCHC) in occupationally-exposed adults (Ukaejiofo et al., 2009; Khan et al., 2008; Patil et al., 2006b; Karita et al., 2005) and in children in association with blood Pb levels (Queirolo et al., 2010; Shah et al., 2010; Olivero-Verbel et al., 2007; Riddell et al., 2007). Several recent toxicological studies also observed decreased Hb levels in laboratory animals exposed to Pb (Sharma et al., 2010b; Baranowska-Bosiacka et al., 2009; Simsek et al., 2009; Marques et al., 2006; Lee et al., 2005). Decreased Hb levels are a direct indicator of decreased heme synthesis due to Pb exposure.

In summary, altered heme synthesis is demonstrated by a small, but coherent, body of studies in adult animals reporting that Pb exposures via drinking water and gavage (resulting in blood Pb levels relevant to this ISA) for 15 days to 9 months decreased ALAD and ferrochelatase activities. Supporting this toxicological evidence is a larger body of ecotoxicological studies that demonstrate decreased ALAD activity across a wide range of taxa exposed to Pb. Epidemiologic studies demonstrate evidence in both adults and children that concurrent blood Pb levels are associated with decreased ALAD and ferrochelatase activities. However, the majority of these studies are limited by the lack of rigorous methodology and consideration for potential confounding. Although there are limitations in the epidemiologic evidence, some studies in children did control for or considered potential confounding and effects in adults and children are coherent with effects observed in exposed animals. Because epidemiologic evidence is limited to

associations with concurrent blood Pb levels, there is uncertainty regarding the timing, duration, magnitude, and frequency of Pb exposure associated with decreased RBC survival and function. Evidence for altered heme synthesis is also provided by a large body of toxicological and epidemiologic studies that report decreased Hb concentrations in association Pb exposure or blood Pb levels. Collectively, the strong evidence from toxicological and ecotoxicological studies, which are supported by findings from epidemiologic studies, are sufficient to conclude that there is a causal relationship between Pb exposures and altered heme synthesis.

Table 4-35 Summary of evidence supporting RBC survival and heme synthesis causal determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	Recent References[b]	Pb Exposure or Blood Pb Levels Associated with Effects[c]
Decreased RBC Survival and Function: Causal			
Consistent evidence in animals with relevant exposures to rule out chance, bias, and confounding with reasonable confidence	Large body of studies with consistent findings for decreased RBC survival and function (decreased Hb, Hct, PVC, increased eryptosis, decreased hematopoiesis, increased oxidative stress) in rodents with relevant concentrations of Pb and routes of exposure	Baranowska-Bosiacka et al. (2009), Lee et al. (2005), Sharma et al. (2010b), Simsek et al. (2009), Marques et al. (2006), Molina et al. (2011), Jang et al (2011), Celik et al. (2005), Alghazal et al. (2008b), Kharoubi et al. (2008b), Gautam and Flora (2010) See Sections 4.7.2.2, 4.7.2.3, and 4.7.2.7	Rodents: Blood Pb level: 1.7-7.1 µg/dL Exposures: Drinking water 50-2,000 ppm, 21-270 days as adults Oral gavage 25-500 mg/kg, 28-70 days
Support from multiple, consistent epidemiologic studies with relevant blood Pb levels Some consider potential confounding.	Cross-sectional studies that considered potential confounding factors found blood Pb-associated decreases in Hb, increases in anemia prevalence, increased oxidative stress in children ages 6 mo-5 yr	Riddell et al. (2007), Queirolo et al. (2010), Ahamed et al. (2007), Ahamed et al. (2008) See Sections 4.7.2.2, and 4.7.2.7	Children: majority of concurrent blood Pb levels <15 µg/dL
	Association with Hb found in children with concurrent blood Pb levels with consideration for potential confounding by age, sex, mouthing behavior, anemia, dairy product consumption, maternal age, education, employment, marital status, family structure, SES-related variables	Queirolo et al. (2010) See Section 4.7.2.2	
	Other studies of Hb, oxidative stress adjusted for factors such as age, sex, birthweight, breastfeeding history, urban/rural residence	Riddell et al. (2007), Ahamed et al. (2008), Ahamed et al. (2007) See Sections 4.7.2.2, and 4.7.2.7	

Table 4-35 (Continued): Summary of evidence supporting RBC survival and heme synthesis causal determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	Recent References[b]	Pb Exposure or Blood Pb Levels Associated with Effects[c]
Support from multiple, consistent epidemiologic studies with relevant blood Pb levels (continued)	Consistent evidence in large body of cross-sectional studies without consideration for potential confounding in occupationally-exposed adults and in children of associations of blood Pb levels with decreases RBC survival, interferes with hematopoiesis, and increases oxidative stress	Karita et al. (2005), Khan et al. (2008), Patil et al. (2006a), Patil et al. (2006b), Ukaejiofo et al. (2009), Conterato et al. (2013), Cabaravdic et al. (2010), Ergurhan-Ilhan et al. (2008), Mohammad et al. (2008), Quintanar-Escorza et al. (2007), Sakata et al. (2007), Riddell et al. (2007), Queirolo et al. (2010), Olivero-Verbel et al. (2007), Ahamed et al. (2006), Ahamed et al. (2007), Ahamed et al. (2008), Turgut et al. (2007), Huo et al. (2007), Shah et al. (2010), Rondo et al. (2006), Jin et al. (2006) See Sections 4.7.2.2, 4.7.2.3, and 4.7.2.7	Adults (occupational exposures): majority of blood Pb levels >20 µg/dL, some studies observed effects in the range of 5–7 µg/dL
Support from evidence that clearly describes mode of action			
Altered RBC membrane ion transport:	Evidence of increased $[Ca^{2+}]_I$ and decreased Ca^{2+}/Mg^{2+}ATPase activity in the RBCs of exposed workers. $[Ca^{2+}]_I$ levels highly correlated with blood Pb even among unexposed controls. $[Ca^{2+}]_I$ levels increased in RBCs from healthy volunteers when exposed in vitro to Pb $[Ca^{2+}]_I$ associated with increased RBC fragility and alterations in RBC morphology	See Section 4.7.2.2	
Phosphatidylserine (PS) expression:	Consistent evidence from in vivo and in vitro studies that Pb exposure increases PS expression on RBC membranes via modulation of $[Ca^{2+}]_I$ concentrations. Increased PS expression leads to eryptosis and phagocytosis by macrophages	See Section 4.7.2.2	

Table 4-35 (Continued): Summary of evidence supporting RBC survival and heme synthesis causal determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	Recent References[b]	Pb Exposure or Blood Pb Levels Associated with Effects[c]
Altered Heme Synthesis: Causal			
Consistent evidence in animals with relevant exposures to rule out chance, bias, and confounding with reasonable confidence	A small, but coherent toxicology database indicates decreased heme synthesis in rodents with relevant Pb concentrations and routes of exposure	Rendón-Ramirez et al. (2007), Lee et al. (2005), Gautam and Flora (2010) See Section 4.7.3.1	Blood Pb levels 6.5 µg/dL Exposures: 500–5,000 ppm Drinking water, 15–30 days as adults
	Consistent eco-toxicological evidence for Pb-induced decreased ALAD activity observed across many taxa (bivalves, fish, amphibians, birds, and mammals) in multiple studies	Birds: Berglund et al. (2010), Gómez-Ramírez et al. (2011), Hansen et al. (2011a), Martinez-Haro et al. (2011) See Section 6.3.4.3	Birds: 6 – >100 µg/dL
		Freshwater Invertebrates: Aisemberg et al. (2005) See Section 6.4.5.2	Freshwater Invertebrates (48-h exposure in aquaria) 0.2-300 µg/g wet tissue
		Fish: Schmitt et al. (2005), Schmitt et al. (2007b), Heier et al. (2009), See Section 6.4.5.3	Fish: 6–14 µg/g (gill or liver concentrations)
		Bivalves: Kalman et al. (2008), Company et al. (2011). See Section 6.4.15.2	Bivalves: 0.38–3.50 µg/g dry weight

Table 4-35 (Continued): Summary of evidence supporting RBC survival and heme synthesis causal determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	Recent References[b]	Pb Exposure or Blood Pb Levels Associated with Effects[c]
Support from multiple, consistent epidemiologic studies with relevant blood Pb levels	Cross-sectional studies that considered potential confounding by age, sex, urban/rural residence, height, weight, BMI found associations with lower ALAD and ferrochelatase activities in children.	Ahamed et al. (2006), Ahamed et al. (2007) See Section 4.7.3.1, Section 4.7.4.1, and Section 4.7.4.2	Adults (occupational exposure) and children: Majority of concurrent blood Pb levels >20 µg/dL, Two studies observed associations in adults and children in the range of concurrent blood Pb levels 6–7 µg/dL.
	Concurrent blood Pb level associated with lower ALAD and higher ZPP in adults with consideration for potential confounding by age, sex, smoking status, and alcohol use.	Wang et al. (2010g) See Sections 4.7.3.1, and 4.7.3.2	
	Associations found in several studies, mostly in occupationally-exposed adults, that did not consider potential confounding	Children: Ahamed et al. (2005) Occupational: Ademuyiwa et al. (2005b), Mohammad et al. (2008), Patil et al. (2006a), (2006b), Quintanar-Escorza et al. (2007), Conterato et al. (2013) See Sections 4.7.3.1, and 4.7.3.2	
Support from evidence for decreases in Hb, a direct marker of decreased heme synthesis	Consistent evidence in animals with relevant Pb exposures for decreases in Hb.	Animals: Baranowska-Bosiacka et al. (2009), Lee et al. (2005), Marques et al. (2006), Sharma et al. (2010b), Simsek et al. (2009) See Section 4.7.2.2	Adult animals: Blood Pb levels 1.7–7.1 µg/dL after 15 day-9 month Pb exposure
	Association found in children between decreased Hb and concurrent blood Pb levels with consideration for potential confounding by age, sex, mouthing behavior, anemia, dairy product consumption, maternal age, education, employment, marital status, family structure, SES-related variables	Queirolo et al. (2010) See Section 4.7.2.2	Children: Majority of concurrent blood Pb <15 µg/dL
	Other studies in children had limited or no consideration for potential confounding.	Shah et al. (2010), Olivero-Verbel et al. (2007), Riddell et al. (2007) See Section 4.7.2.2	

Table 4-35 (Continued): Summary of evidence supporting RBC survival and heme synthesis causal determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	Recent References[b]	Pb Exposure or Blood Pb Levels Associated with Effects[c]
Support from evidence for decreases in Hb, a direct marker of decreased heme synthesis (continued)	Associations found in adults and, as well as coherent findings in animal toxicological studies, for decreased Hb.	Adults: Karita et al. (2005), Khan et al. (2008), Patil et al. (2006b), Ukaejiofo et al. (2009) See Section 4.7.2.2	Adults (occupational exposure): Majority of blood Pb >20 µg/dL
Support from evidence that describes mode of action	Altered Ion Status: Evidence that Pb competitively inhibits the binding of Zn ions necessary for ALAD activity. Pb also inhibits the incorporation of Fe into protoporphyrin IX by ferrochelatase, resulting in Zn-protoporphyrin production	See Sections 4.2.2.3. 4.2.2.4, and 4.2.4.1	

[a]Described in detail in Table II of the Preamble.

[b]Describes the key evidence and references contributing most heavily to causal determination and where applicable to uncertainties or inconsistencies. References to earlier sections indicate where the full body of the evidence is described.

[c]Describes the blood Pb levels in humans with which the evidence is substantiated and blood Pb levels in animals most relevant to this ISA.

4.8 Reproductive and Developmental Effects

The effect of Pb on reproductive and developmental outcomes has been of interest for years, starting in cohorts of occupationally-exposed individuals. More recently, researchers have begun to focus on reproductive and developmental effects in populations without occupational exposures, with more environmentally-relevant levels of Pb exposure. The toxicological and epidemiologic literature on reproductive effects of Pb includes research on female and male reproductive function such as hormone levels, fertility, spontaneous abortions, effects on sperm, estrus, and effects on reproductive organs. Evaluation of effects on development includes effects on puberty onset, postnatal growth, and effects on the development of the teeth, sensory organs, and other systems. Research on birth outcomes includes birth defects, infant mortality, preterm birth, and low birth weight. A few studies of pregnancy-induced hypertension and eclampsia have been conducted and are reported on in the section on hypertension (Section 4.4.2.1). Briefly, the relatively small number of studies found consistently positive associations between blood Pb levels and pregnancy-induced hypertension. Biomarkers of Pb exposure, including blood Pb and bone Pb, are used in the epidemiologic studies reviewed in this section. Bone Pb typically indicates cumulative exposure to Pb, whereas, blood Pb may indicate more recent exposure. However, Pb can also be remobilized from the bone during times of active bone remodeling, such as pregnancy or lactation. Therefore, blood Pb also may reflect cumulative Pb exposure. Toxicological studies typically report exposure using blood Pb. More detailed discussion of these measures and Pb transfer via umbilical cord blood Pb across the placenta, and via lactation is given in Section 3.2.2.4 on Pb Toxicokinetics.

Overall, the recent literature on reproductive effects of Pb exposure continues to support associations reported in earlier Pb AQCDs between Pb exposure and effects on various parameters of sperm (function, motility, count, integrity, histology). The toxicological and epidemiologic literature of developmental effects of Pb exposure also indicates that Pb exposure is associated with delayed onset of puberty in both males and females. Associations between Pb exposure and other reproductive and developmental effects have less consistent findings. The recent information from epidemiologic and toxicological studies is integrated with conclusions from previous Pb AQCDs.

4.8.1 Effects on Development

The 2006 Pb AQCD (U.S. EPA, 2006b) reported Pb-associated developmental effects on teeth, sensory organs, the GI system, the liver, and postnatal growth as well as delayed

puberty onset (U.S. EPA, 2006b). There was recognition that Pb is transferred across the placenta and through the breast milk, contributing to exposure during development. The 2006 Pb AQCD reported delayed puberty onset in both male and female populations in animal toxicology studies showing associations with dam blood Pb levels of ~40 µg/dL and pup blood Pb levels of 26 µg/dL. The research reported in this ISA continues to find delayed puberty onset with Pb exposure at even lower Pb doses in animal toxicology studies as is detailed below. Mechanistic understanding of delayed puberty onset is also reported in this ISA. Lower dose Pb exposure studies in animal toxicology are also reported in studies of retinal function and postnatal growth in this ISA. Studies included in this ISA expand upon evidence reported in previous Pb AQCDs for the aforementioned systems sensitive to developmental effects with recent studies showing effects at lower doses of Pb. This section does not cover associations between Pb and neurodevelopmental impacts, which are discussed in detail in Section 4.3. The studies presented in the following text and tables are grouped by study design and methodological strength.

4.8.1.1 Effects on Puberty among Females

Recent toxicological studies of rodents have examined the effects of Pb on pubertal and reproductive organ development and on biomarkers of pubertal development among females. There have also been recent epidemiologic studies examining associations between blood Pb levels and onset of puberty among girls, which are summarized in Table 4-36 and in the text below. All of the epidemiologic studies examined concurrently measured blood Pb and puberty and are reported below. Additionally, while there was a longitudinal investigation by Naicker et al. (2010), who followed girls to determine their age of menarche, blood Pb levels were measured once at 13 years of age.

Table 4-36 Summary of epidemiologic studies of associations between blood Pb levels and puberty for females.

Reference[a]	Outcome	Study Population Study Location Study Years	Methodological Details	Blood Pb Data in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Selevan et al. (2003)	Tanner staging and age at menarche	Girls ages 8-18 yr from the NHANES III study $N_{NH\ White}$=600 $N_{NH\ Black}$=805 $N_{Mexican\text{-}American}$=781 U.S.A. 1988-1994	Cross-sectional study using ordinal logistic regression and Cox proportional hazards	Geometric mean Non-Hispanic (NH) Whites: 1.4 NH Blacks: 2.1 Mexican-Americans: 1.7 Blood Pb levels>5µg/dL: NH Whites: 2.7% NH Blacks: 11.6% Mexican-Americans: 12.8% Blood Pb levels >10 µg/dL: NH Whites: 0.3% NH Blacks: 1.6% Mexican-Americans: 2.3%	OR (95% CI) Breast development NH Whites 1 µg/dL: 1.00 (Ref) 3 µg/dL: 0.82 (0.47, 1.42) NH Blacks: 1 µg/dL: 1.00 (Ref) 3 µg/dL: 0.64 (0.42, 0.97) Mexican Americans: 1 µg/dL: 1.00 (Ref) 3 µg/dL: 0.76 (0.63, 0.91) Pubic hair development NH Whites: 1 µg/dL: 1.00 (Ref) 3 µg/dL: 0.75 (0.37, 1.51) NH Blacks: 1 µg/dL: 1.00 (Ref) 3 µg/dL: 0.62 (0.41, 0.96) Mexican Americans: 1 µg/dL: 1.00 (Ref) 3 µg/dL: 0.70 (0.54, 0.91)	For breast development: Age, age^2, height, BMI, family income, ever smoked>100 cigarettes, dietary Fe, dietary vitamin C, dietary Ca^{2+}. For pubic hair development: Age, age^2, height, family income, ever smoked>100 cigarettes, anemia, dietary Fe, dietary vitamin C Considered in all models: age, smoking, dietary Ca^{2+}, dietary Fe, dietary vitamin C, dietary total fat, anemia, urban residence, family income

Table 4-36 (Continued): Summary of epidemiologic studies of associations between blood Pb levels and puberty for females.

Reference[a]	Outcome	Study Population Study Location Study Years	Methodological Details	Blood Pb Data in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Selevan et al. (2003) (Continued)	Tanner staging and age at menarche	Girls ages 8-18 yr from the NHANES III study $N_{NH\ White}$=600 $N_{NH\ Black}$=805 $N_{Mexican\text{-}American}$=781 U.S.A. 1988-1994	Cross-sectional study using ordinal logistic regression and Cox proportional hazards	Geometric mean Non-Hispanic (NH) Whites: 1.4 NH Blacks: 2.1 Mexican-Americans: 1.7 Blood Pb levels>5µg/dL: NH Whites: 2.7% NH Blacks: 11.6% Mexican-Americans: 12.8% Blood Pb levels >10 µg/dL: NH Whites: 0.3% NH Blacks: 1.6% Mexican-Americans: 2.3%	**HR (95% CI)** *included only girls ages 8-16 yr Age at menarche NH Whites: 1 µg/dL: 1.00 (Ref) 3 µg/dL: 0.74 (0.55, 1.002) NH Blacks: 1 µg/dL: 1.00 (Ref) 3 µg/dL: 0.78 (0.63, 0.98) Mexican Americans: 1 µg/dL: 1.00 (Ref) 3 µg/dL: 0.90 (0.73, 1.11)	For age at menarche: Height, BMI, family income, anemia, dietary Ca^{2+}. Considered in all models: age, smoking, dietary Ca^{2+}, dietary Fe, dietary vitamin C, dietary total fat, anemia, urban residence, family income

Table 4-36 (Continued): Summary of epidemiologic studies of associations between blood Pb levels and puberty for females.

Reference[a]	Outcome	Study Population Study Location Study Years	Methodological Details	Blood Pb Data in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Wu et al. (2003b)	Tanner staging and age at menarche	Girls ages 8-16 yr from the NHANES III study N=1706 U.S.A. 1988-1994	Cross-sectional study using logistic regression with weighting	Mean (SD): 2.5 (2.2) Weighted proportion of the sample with blood Pb 5.0-21.7: 5.9%	OR (95% CI) Breast development 0.7-2.0 µg/dL: 1.00 (Ref) 2.1-4.9 µg/dL: 1.51 (0.90, 2.53) 5.0-21.7 µg/dL: 1.20 (0.51, 2.85) Pubic hair development 0.7-2.0 µg/dL: 1.00 (Ref) 2.1-4.9 µg/dL: 0.48 (0.25, 0.92) 5.0-21.7 µg/dL: 0.27 (0.08, 0.93) Menarche 0.7-2.0 µg/dL: 1.00 (Ref) 2.1-4.9 µg/dL: 0.42 (0.18, 0.97) 5.0-21.7 µg/dL: 0.19 (0.08, 0.43)	Race/ethnicity, age, family size, residence, poverty income, ratio, BMI

Table 4-36 (Continued): Summary of epidemiologic studies of associations between blood Pb levels and puberty for females.

Reference[a]	Outcome	Study Population Study Location Study Years	Methodological Details	Blood Pb Data in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Gollenberg et al. (2010)	Luteinizing hormone (LH) and inhibin B	Girls ages 6-11 yr from the NHANES III study N=705 U.S.A. 1988-1994	Cross-sectional study using survey logistic regression	Median 2.5 (range 0.07-29.4) Blood Pb >10 µg/dL: 5%	OR (95% CI) Exceeding pubertal inhibin B cutoff (>35pg/mL) <1 µg/dL: 1.00 (Ref) 1-4.9 µg/dL: 0.38 (0.12, 1.15) ≥ 5 µg/dL: 0.26 (0.11, 0.60) Exceeding pubertal LH cutoff (>0.4 mIU/mL) <1 µg/dL: 1.00 (Ref) 1-4.9 µg/dL: 0.98 (0.48, 1.99) ≥ 5 µg/dL: 0.83 (0.37, 1.87) *Note: a sensitivity analysis including only those with blood Pb <10 µg/dL had similar results but ORs were slightly attenuated	Age, race/ethnicity, BMI, census region, poverty-income ratio

Table 4-36 (Continued): Summary of epidemiologic studies of associations between blood Pb levels and puberty for females.

Reference[a]	Outcome	Study Population Study Location Study Years	Methodological Details	Blood Pb Data in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Denham et al. (2005)	Age at menarche	10- to 16.9-yr-old girls in the Akwesasne community N=138 Akwesasne Mohawk Nation (boundaries of New York, Ontario, and Quebec Years not reported	Cross-sectional study using probit and logistic regression	Mean (SD): 0.49 (0.905) Median: 1.2	Coefficients for binary logistic regression predicting menarche with Pb centered at the mean: **log blood Pb** -1.29 (p-value 0.01) **log blood Pb -squared**: -1.01 (p-value 0.08) Non-linear relationship observed and Pb below the mean did not appear to affect the odds of menarche. Increasing blood Pb from 0.49 to 0.98 µg/dL decreased the odds of menarche attainment by 72%	Age, SES, BMI
Naicker et al. (2010)	Self-reported Tanner staging at age 13 and age at menarche	Girls of black or mixed ancestry who were enrolled in the Birth to Twenty (Bt20) cohort (born in 1990) that lived in Johannesburg/ Soweto for at least 6 mo after birth N=682 Johannesburg/ Soweto, South Africa Born in 1990	Cross-sectional and longitudinal study using logistic regression	Mean (SD): 4.9 (1.9) Blood Pb levels ≥ 10 µg/dL: 1%	OR (95% CI) Delay in breast development at age 13 <5 µg/dL: 1.00 (Ref) ≥ 5 µg/dL: 2.34 (1.45, 3.79) Delay in pubic hair development at age 13 <5 µg/dL: 1.00 (Ref) ≥ 5 µg/dL: 1.81 (1.15, 2.84) Delay in attainment of menarche at age 13 <5 µg/dL: 1.00 (Ref) ≥ 5 µg/dL: 2.01 (1.38, 2.94)	BMI

Table 4-36 (Continued): Summary of epidemiologic studies of associations between blood Pb levels and puberty for females.

Reference[a]	Outcome	Study Population Study Location Study Years	Methodological Details	Blood Pb Data in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Den Hond et al. (2011)	Tanner staging, age at menarche, regular menses	Girls ages 14 and 15 yr, in their 3rd year of secondary education and living in the same study areas for at least 5 years N=792 Flanders, Belgium 2003-2004	Cross-sectional study using logistic regression	Median: 1.81 10th percentile: 0.88 90th percentile: 3.81	OR (95% CI) Pubic hair development with doubling of exposure: 0.65 (0.45, 0.93) *Association was no longer statistically significant when PCB marker included in the model No association between Pb and breast development (results not given)	Age, BMI, smoking, oral contraceptive use Considered but did not include: food intake and lifestyle parameters

Table 4-36 (Continued): Summary of epidemiologic studies of associations between blood Pb levels and puberty for females.

Reference[a]	Outcome	Study Population Study Location Study Years	Methodological Details	Blood Pb Data in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Tomoum et al. (2010)	Hormones and pubertal development using Tanner staging	Healthy children aged 10-13 yr; seeking treatment for minor health problems and living in one of two designated areas (one with high-risk for Pb contamination and one with no Pb source) N=20 Cairo, Egypt 2007	Cross-sectional study using Chi-square	Blood Pb Not stated for girls only (combined with boys in the study the mean was 9.46 [3.08])	**Breast Development** **<10 µg/dL:** Stage 2: 36.4% Stage 3: 63.6% **≥ 10 µg/dL:** Stage 2: 100% Stage 3: 0% Chi-square p-value<0.01 **Pubic Hair Development** **<10 µg/dL:** Stage 2: 36.4% Stage 3: 63.6% **≥ 10 µg/dL:** Stage 2: 77.8% Stage 3: 22.2% Chi-square p-value>0.05	None

Table 4-36 (Continued): Summary of epidemiologic studies of associations between blood Pb levels and puberty for females.

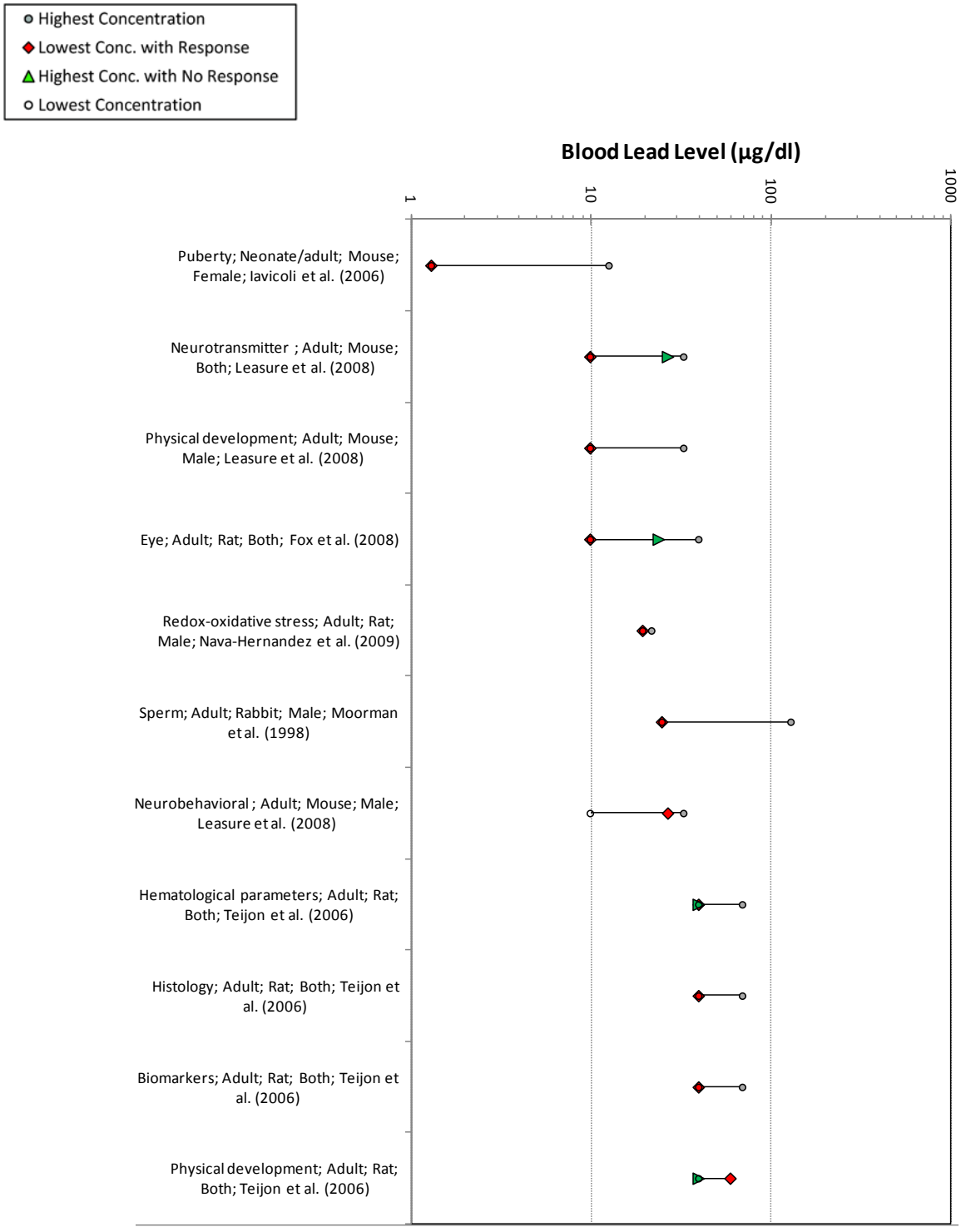
Reference[a]	Outcome	Study Population Study Location Study Years	Methodological Details	Blood Pb Data in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Tomoum et al. (2010) (Continued)	Hormones and pubertal development using Tanner staging	Healthy children aged 10-13 yr; seeking treatment for minor health problems and living in one of two designated areas (one with high-risk for Pb contamination and one with no Pb source) N=20 Cairo, Egypt 2007	Cross-sectional study using Chi-square	Blood Pb Not stated for girls only (combined with boys in the study the mean was 9.46 [3.08])	**Median FSH level** **<10 µg/dL:** 7.3 (IQR 7.9) mIU/mL **≥ 10 µg/dL:** 3.17 (IQR 2.6) mIU/mL Mann-Whitney U-test p-value <0.05 **Median LH level** **<10 µg/dL:** 8.9 (IQR 6.1) mIU/mL **≥ 10 µg/dL:** 1.23 (IQR 2.5) mIU/mL Mann-Whitney U-test p-value<0.05 Median Estradiol levels were similar among girls with blood Pb levels of <10 µg/dL and ≥ 10 µg/dL (quantitative results not given).	None
Wolff et al. (2008); Wolf and Daley (2007)	Pubertal stages defined using standard drawings	9-yr old girls from the study hospital and nearby pediatric offices N=192 New York City, NY 1996-1997	Cross-sectional study using Poisson multivariate regression with robust error variance	Median: 2.4	**PR (95% CI)** (unit not given, assume results are per 1 µg/dL) **Breast stage**: 1.01 (0.79, 1.30) **Pubic hair stage:** 1.25 (0.83, 1.88)	For breast development: Age, BMI, race For hair stage: Height, private clinic, race

[a]Studies are presented in order of first appearance in the text of this section, which is based on study design and methodological strength.

Multiple studies have been performed examining blood Pb levels and puberty using NHANES III data (Gollenberg et al., 2010; Selevan et al., 2003; Wu et al., 2003b). A study included girls 8-18 years of age and reported the results stratified by race (Selevan et al., 2003). This study also included many important potential confounders, such as nutritional factors. Higher blood Pb levels were associated with lower Tanner stage of breast and pubic hair development and later age at menarche among African Americans and with lower stage of breast and pubic hair development among Mexican Americans. For whites, the associations were in the same directions, but none reached statistical significance. Another NHANES III study included girls aged 8-16 years and did not stratify the results by race/ethnicity (Wu et al., 2003b). The study, which also controlled for a variety of potential confounders (although not nutritional factors), reported an association between increased blood Pb and delayed attainment of menarche and pubic hair development but not breast development. The associations were observed even at blood Pb levels of 2.1-4.9 µg/dL compared to girls with blood Pb levels <2.1 µg/dL. In a study of girls aged 6-11 years old from NHANES III data, higher blood Pb levels were associated with lower inhibin B, a protein that inhibits follicle stimulating hormone (FSH) production, but no association was observed for luteinizing hormone (LH). (Gollenberg et al., 2010). The inverse association between blood Pb and inhibin B was greater among girls with Fe deficiency compared to those with high Pb but sufficient Fe levels. Inhibin B and LH were chosen for this study because, as the authors indicated, these hormones are, "believed to be relevant for younger girls… near the onset of puberty and…serve as markers for hypothalamic-pituitary-gonadal functioning."

A study of girls aged 10-16.9 years of age in the Akwesasne Mohawk Nation reported a nonlinear association between higher blood Pb and greater age at menarche (Denham et al., 2005). No association was observed below blood Pb of 0.49 µg/dL in a nonlinear model of the Pb-menarche relationship. A study conducted in South Africa reported an association between increased blood Pb levels and older age at first menarche and pubertal development (Naicker et al., 2010). Another study reporting on girls with low blood Pb concentrations observed an association between higher blood Pb and less pubic hair development but not breast development (Den Hond et al., 2011). The association was no longer statistically significant when a marker for polychlorinated biphenyl exposure was included in the model. A study among girls aged 10-13 years (median: 12 years) reported lower levels of FSH and LH levels in the group with blood Pb of at least 10 µg/dL compared to the group with blood Pb less than 10 µg/dL (Tomoum et al., 2010). In addition, there were some indications of lower Tanner stages of breast development associated with Pb levels of at least 10 µg/dL, but this relationship was not present for stages of pubic hair development and there was no control for potential confounders. A study performed in NYC among 9-year old girls reported no association

between Pb levels and pubertal development (Wolff et al., 2008), but this age group may be too young to study when investigating delayed puberty as the outcome.



Note: This figure illustrates reproductive and developmental effects associated with Pb exposure in studies that examined multiple exposure concentrations. Dosimetric representation reported by blood Pb level. (Studies are described in Table 4-37).

Figure 4-36 Toxicological concentration-response array for reproductive and developmental effects of Pb.

Table 4-37 Toxicological concentration-response array summary for reproductive and developmental effects of Pb presented in Figure 4-36.

Reference	Exposure	Blood Pb level with Effect (µg/dL)	Altered Outcome
Iavicoli et al. (2006a)	Lifetime Pb exposure starting in utero. Dam exposure during gestation (0.2- 40 ppm daily dietary Pb); offspring continuous dietary exposure until the termination of the experiment at puberty	1.3 & 13	Delayed onset female puberty
Leasure et al. (2008)	Gestational and Lactational Exposure to one of 3 Pb acetate doses (27, 55, or 109 ppm) in dam drinking water, 2 weeks before mating, through gestation out to PND10.	10 & 42	Neurotransmitter, Dopamine homeostasis
		10, 24 & 42	Physical Development, Adult obesity (males)
		10 & 42	Aberrant response to amphetamine
Fox et al. (2008)	Dam exposure 2 weeks before mating, throughout gestation, and until PND10 (27, 55, or 109 ppm Pb acetate in drinking water)	12	Retinal aberrations
Nava-Hernandez et al. (2009)	Adult male rats, 10 mg/kg BW Pb chloride i.p. once daily for 8 weeks	19.5	Sperm affected via redox imbalance
Moorman et al. (1998)	Adult male rabbits, chronic exposure: subcutaneous injection, loading dose of 0.2-3.85 mg/kg BW Monday (M), Wednesday (W) and Fridays (F) weeks 6-10, followed by maintenance dose of 0.13-2.0 mg/kg BW Pb acetate MWF over weeks 11-20 of the study	25-130	Semen quality affected
Teijon et al. (2006)	Wistar rats, 200 ppm or 400 ppm Pb acetate in drinking water to dams from GD1 through lactation & 1 and 3 months postweaning to pups	40 & 100	Hematology
		40 & 100	Histology: Offspring renal & hepatic
		40 & 100	Biomarker: Offspring renal function
		100	Physical development: Birth weight

Table 4-37 (Continued): Toxicological concentration-response array summary for reproductive and developmental effects of Pb presented in Figure 4-36.

Reference	Exposure	Blood Pb level with Effect (µg/dL)	Altered Outcome
Fox et al. (2008)	Dam exposure 2 weeks before mating, throughout gestation, and until PND10 (27, 55, or 109 ppm Pb acetate in drinking water)	12	Retinal aberrations
Nava-Hernandez et al. (2009)	Male adult albino rats, 10 mg/kg BW Pb chloride i.p. once daily for 8 weeks	19.5	Sperm affected via redox imbalance
Moorman et al. (1998)	Adult male rabbits, chronic exposure: subcutaneous injection, loading dose of 0.2-3.85 mg/kg BW Monday (M), Wednesday (W) and Fridays (F) weeks 6-10, followed by maintenance dose of 0.13-2.0 mg/kg BW Pb acetate MWF over weeks 11-20 of the study	25-130	Semen quality affected
Teijon et al. (2006)	Wistar rats, 200 ppm or 400 ppm Pb acetate in drinking water to dams from GD1 through lactation & 1 and 3 months postweaning to pups	40 & 100	Hematology
		40 & 100	Histology: Offspring renal & hepatic
		40 & 100	Biomarker: Offspring renal function
		100	Physical development: Birth weight
Fox et al. (2008)	Dam exposure 2 weeks before mating, throughout gestation, and until PND10 (27, 55, or 109 ppm Pb acetate in drinking water)	12	Retinal aberrations

Earlier studies showed that prenatal and lactational exposures to Pb can cause a delay in the onset of female puberty in rodents. Recent studies corroborate these findings and show that puberty onset is one of the more sensitive markers of reproductive or developmental effects of Pb exposure as is demonstrated in the exposure response array (Figure 4-36 and Table 4-37; including outcomes described in sections that follow). Dumitrescu et al. (2008b) exposed adult Wistar female rats to varying doses of Pb acetate (50-150 ppb) in drinking water for 3 months before mating and during pregnancy. Vaginal opening, an indicator of sexual maturation, was statistically significantly delayed in pups from all Pb treated groups when compared to pups from non-treated dams. The age at vaginal opening in female pups from the Pb treated groups increased, in a concentration-dependent manner, from 39 days to 43-47 days. The authors also observed

a correlation between body weight and age at vaginal opening meaning that as body weight decreased the age at vaginal opening increased. This effect also exhibited a concentration-dependent relationship.

In recent studies by one lab, Iavicoli et al. (Iavicoli et al., 2006a; 2004) reported a statistically significant delay in several indicators of sexual maturity in female offspring (Swiss mice, F1 generation) born to dams that ingested 0.02- 40 ppm Pb in their daily diet during pregnancy; offspring had continuous dietary exposure until the termination of the experiment at puberty. Maternal ingestion of Pb at the various doses resulted in female pup blood Pb levels of 0.7, 1.3, 1.6, 1.9, 3.5, 3.9, 8 and 13 µg/dL. There was a dose-dependent delay in age at vaginal opening, age of first estrus, age of vaginal plug formation, and age of first parturition . Vaginal opening was significantly different between the following groups at blood Pb levels of 0.7, 1.3, 1.6, 1.9-3.9, 8 and 13 µg/dL. Estrus cyclicity, age of first vaginal plug detection and age of first parturition were significantly different between the groups with blood Pb levels of 0.7, 1.3-1.6, 1.9-3.9, 8 and 13 µg/dL. After in utero Pb exposure, sexual development was delayed in a concentration-dependent fashion in Pb-exposed female offspring.

In addition, Pb-induced shifts in sexual maturity were observed in the subsequent generation (F_2 generation) across the same dose range (Iavicoli et al., 2006a). These F_2 animals continued to be exposed to the aforementioned concentrations of Pb over multiple generations through the diet. Results in the F_2 generation including blood Pb levels and markers of delayed sexual maturity in female offspring closely resembled those of the F_1 generation.

In the 2006 Pb AQCD (U.S. EPA, 2006b), it was reported that a statistically significant reduction in the circulating levels of insulin-like growth factor 1 (IGF-1), LH, and estradiol (E2) was associated with Pb-induced delayed puberty onset in Fischer 344 pups, providing evidence for the effect of Pb on a mode of action for delayed puberty onset. Subsequently, Pine et al. (2006) evaluated whether IGF-1 replacement could reverse the effects of Pb on delayed female puberty onset, providing more evidence for the effect on a mode of action associated with Pb on delayed puberty onset. The authors reported that offspring from dams exposed to Pb during gestation and lactation (daily oral gavage of dam with 1.0 mL solution of Pb acetate 12 mg/mL; mean maternal blood Pb level 40 µg/dL) exhibited a marked increase in LH and luteinizing hormone releasing hormone (LHRH) secretion after IGF-1 administration (200 ng^3/µL i.p. injection twice daily from PND23 until the appearance of vaginal opening which appears in control animals at ~PND40) resulting in restored timing of vaginal opening to that of control animals. It should be noted that, IGF-1 replacement in Pb-exposed animals did not cause advanced puberty onset over non-Pb-exposed controls. The results of this study provide support to

the theory that Pb-induced delayed onset of puberty may be due to disruption of pulsatile release of sex hormones (U.S. EPA, 2006b) and not necessarily due to a direct toxic effect on the hypothalamic-pituitary-gonadal axis (Salawu et al., 2009), and IGF-1 may play a prominent role in the process.

In summary, epidemiologic studies consistently show an association between higher concurrent blood Pb and delayed pubertal development in girls. This association is apparent even at low blood Pb levels (mean and/or median concurrent blood Pb levels of 1.2 to 9.5 µg/dL). Most of the studies had good sample sizes and controlled for some potential confounders. Nutritional information was rarely controlled for although this could be important, especially in populations where malnutrition is prevalent. These epidemiologic studies are cross-sectional, which does not allow for the study of temporality between Pb exposure and pubertal onset nor does it consider the influence of past blood Pb levels. Recent evidence from the toxicology literature continues to indicate Pb-induced delays in the onset of puberty. Further, the biological plausibility of delayed puberty is expanded with the toxicological literature that shows this pathway is mediated by IGF-1.

4.8.1.2 Effects on Puberty among Males

Recent epidemiologic studies examining the association between blood Pb and onset of puberty in males are summarized in Table 4-38. The majority of studies used concurrent measures of blood Pb and puberty (Den Hond et al., 2011; Tomoum et al., 2010; Hauser et al., 2008), but Williams et al. (2010) performed a longitudinal analysis of blood Pb levels measured at ages 8-9 years and pubertal onset, following the participants for 3 years. Little epidemiologic information was available regarding pubertal onset in the 2006 Pb AQCD (U.S. EPA, 2006b).

Table 4-38 Summary of recent epidemiologic studies of associations between blood Pb levels and puberty for males.

Reference[a]	Outcome	Study Population Study Location Study Years	Methodological Details	Blood Pb Data in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Hauser et al. (2008)	Pubertal stages defined using standard drawings	Healthy boys aged 8-9 yr N=489 Chapaevsk, Russia 2003-2005	Cross-sectional study using multivariable logistic regression	Median: 3 (IQR 2-5) Blood Pb >10 µg/dL: 3%	OR (95% CI) **Pubertal onset based on testicular volume** **<5 µg/dL**: 1.00 (Ref) **≥ 5 µg/dL:** 0.83 (0.43, 1.59) *after adjustment for macronutrients, the OR (95% CI) became 0.66 (0.44, 1.00) **Genital development** **<5 µg/dL**: 1.00 (Ref) **≥ 5 µg/dL**: 0.57 (0.34, 0.95) *after adjustment for macronutrients, the OR (95% CI) became 0.52 (0.31, 0.88) **Pubic hair development** **<5 µg/dL**: 1.00 (Ref) **≥ 5 µg/dL**: 0.74 (0.34, 1.60)	Gestational age, height, BMI, age at exam Considered but did not include: parental education, household income

Table 4-38 (Continued): Summary of recent epidemiologic studies of associations between blood Pb levels and puberty for males.

Reference[a]	Outcome	Study Population Study Location Study Years	Methodological Details	Blood Pb Data in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Williams et al. (2010)	Pubertal stages defined using standard drawings	Healthy boys aged 8-9 yr at enrollment who had 3 annual follow-up evaluations N=481 Chapaevsk, Russia 2003-2008	Longitudinal cohort using Cox proportional hazards	Blood Pb at ages 8-9 yr Median: 3 (IQR 2-5) Blood Pb level > 10 µg/dL: 3%	HR (95% CI) **Pubertal onset based on testicular volume** **<5 µg/dL**: 1.00 (Ref) **≥ 5 µg/dL:** 0.73 (0.55, 0.97) **Genital development** **<5 µg/dL:** 1.00 (Ref) **≥ 5 µg/dL**: 0.76 (0.59, 0.98) **Pubic hair development** **<5 µg/dL**: 1.00 (Ref) **≥ 5 µg/dL**: 0.69 (0.44, 1.07)	Birthweight, gestational age, energy intake, proportion of fat consumption, proportion of protein consumption, maternal alcohol consumption during pregnancy, height at study entry, BMI at study entry, household income, parental education NOTE: exclusion of BMI and height, in case they were part of the causal pathway, resulted in very similar estimates Considered but not included: parity, maternal or household smoking during pregnancy, maternal age at birth

Table 4-38 (Continued): Summary of recent epidemiologic studies of associations between blood Pb levels and puberty for males.

Reference[a]	Outcome	Study Population Study Location Study Years	Methodological Details	Blood Pb Data in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Tomoum et al. (2010)	Hormones and pubertal development using Tanner staging	Healthy children aged 10-13 yr seeking treatment for minor health problems and living in one of two designated areas (one with high-risk for Pb contamination and one with no Pb source) N=21 Cairo, Egypt 2007	Cross-sectional study using Chi-square	Not stated for boys only (combined with girls in the study the mean was 9.46 [3.08])	**Testicular size** **<10 µg/dL:** Stage 1: 0% Stage 2: 44.4% Stage 3: 55.6% **≥ 10 µg/dL:** Stage 1: 33.3% Stage 2: 66.7% Stage 3: 0% Chi-square p-value<0.01 **Pubic Hair Development** **<10 µg/dL:** Stage 1: 0% Stage 2: 55.6% Stage 3: 44.4% **≥ 10 µg/dL:** Stage 1: 33.3% Stage 2: 66.7% Stage 3: 0% Chi-square p-value<0.05	None

Table 4-38 (Continued): Summary of recent epidemiologic studies of associations between blood Pb levels and puberty for males.

Reference[a]	Outcome	Study Population Study Location Study Years	Methodological Details	Blood Pb Data in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Tomoum et al. (2010) (Continued)	Hormones and pubertal development using Tanner staging	Healthy children aged 10-13 yr seeking treatment for minor health problems and living in one of two designated areas (one with high-risk for Pb contamination and one with no Pb source) N=21 Cairo, Egypt 2007	Cross-sectional study using Chi-square	Not stated for boys only (combined with girls in the study the mean was 9.46 [3.08])	**Penile staging** **<10 µg/dL:** Stage 1: 11.1% Stage 2: 44.4% Stage 3: 44.4% **≥ 10 µg/dL:** Stage 1: 58.3% Stage 2: 41.7% Stage 3: 0% Chi-square p-value<0.05 **Mean testosterone level** **<10 µg/dL:** 4.72 (SD 1.52) ng/mL **≥ 10 µg/dL:** 1.84 (SD 1.04) ng/mL Student t-test p-value <0.05	None

Table 4-38 (Continued): Summary of recent epidemiologic studies of associations between blood Pb levels and puberty for males.

Reference[a]	Outcome	Study Population Study Location Study Years	Methodological Details	Blood Pb Data in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Tomoum et al. (2010) (Continued)	Hormones and pubertal development using Tanner staging	Healthy children aged 10-13 yr seeking treatment for minor health problems and living in one of two designated areas (one with high-risk for Pb contamination and one with no Pb source) N=21 Cairo, Egypt 2007	Cross-sectional study using Chi-square	Not stated for boys only (combined with girls in the study the mean was 9.46 [3.08])	**Median FSH level** **<10 µg/dL**: 5.6 (IQR 7.6) mIU/mL **≥ 10 µg/dL**: 1.88 (IQR 1.4) mIU/mL Mann–Whitney U-test p-value <0.05 **Median LH level** **<10 µg/dL:** 6.5 (IQR 5.8) mIU/mL **≥ 10 µg/dL:** 0.79 (IQR 1.0) mIU/mL Mann–Whitney U-test p-value <0.05	None
Den Hond et al. (2011)	Tanner staging and gyneco-mastia	Boys ages 14 and 15 yr, in their 3rd year of secondary education and living in the same study areas for at least 5 years N=887 Flanders, Belgium 2003-2004	Cross-sectional study using logistic regression	Blood Pb Median: 2.50 10th percentile: 1.20 90th percentile: 5.12	OR (95% CI) for **Gynecomastia with doubling of exposure** 1.84 (1.11, 3.05) No association between Pb and pubic hair or genital development (results not given)	Parental education, age, BMI, smoking status Considered but not included: food intake, lifestyle parameters NOTE: results were the same when hexachlorobenzene was included in the model

[a]Studies are presented in order of first appearance in the text of this section, which is based on study design and methodological strength.

Studies were performed among a cohort of Russian boys enrolled between ages 8-9 years (Williams et al., 2010; Hauser et al., 2008). The area where these studies were performed had various environmental contaminants that may be associated with delayed puberty onset, such as dioxin, polychlorinated biphenyls, and other metals, present but these were not included in the analyses (although preliminary analyses found no correlation between blood Pb levels and serum dioxin levels). Both the cross-sectional study (Hauser et al., 2008) and the prospective study with annual follow-ups (Williams et al., 2010) demonstrated an association; higher blood Pb levels at 8-9 years of age was associated with later onset of puberty. In a study of boys in Egypt, boys with higher blood Pb had delayed pubertal onset compared to those with lower levels (Tomoum et al., 2010). In addition, compared to the low blood Pb group, those boys with higher blood Pb had lower testosterone, FSH, and LH levels but there was no control for potential confounding. A study in Flanders reported no associations between blood Pb concentration and pubertal development among 14- and 15-year old boys (Den Hond et al., 2011). However, higher blood Pb levels were associated with an increased odds of gynecomastia.

No recent toxicological studies address Pb-induced male sexual maturation and development, but earlier studies do provide support to findings in epidemiologic cohorts. Pb exposure resulted in delayed sexual maturity as measured by prostate weight in male Sprague-Dawley pups at PND35. These pups were exposed chronically to 1,500 or 4,500 ppm Pb acetate in dam or their own drinking water from GD5 until PND85 and had blood Pb ranges from low to high of 88-196 and 120-379 μg/dL, respectively (Ronis et al., 1998b). Cynomolgus monkeys exposed to Pb over a lifetime (an oral capsule of 1,500 μg/kg body weight/day for 10 years, blood Pb levels ranging from 30–60 μg/dL) had altered pituitary and Sertoli cell function along with decreases in inhibin/FSH ratio and reduced gonadotropin-releasing hormone (GnRH) stimulation of LH release in adulthood (Foster et al., 1993), all indicators that are important in proper sexual maturation. Further mechanistic understanding of the effect of Pb can be gleaned from studies in adult male Wistar rats exposed to Pb for 1 month (starting at PND56, 1,000 or 3,000 ppm Pb acetate in drinking water, respective blood Pb levels of 34 or 60 μg/dL) that showed significant decreases in FSH, ventral prostate weight and serum testosterone but no change in serum LH (Sokol et al., 1985). These Pb-exposed adult male rats (3,000 ppm Pb acetate in drinking water starting at PND56 for 30 days) demonstrated an impaired pituitary release of LH in response to challenge of the hypothalamic–pituitary–adrenal (HPA) axis with the opiate antagonist naloxone, an enhanced release of LH from the pituitary in response to direct stimulation of the pituitary with luteinizing hormone-releasing hormone (LHRH), an enhanced response to human chorionic gonadotropin (hCG) by the testes, increased pituitary LH stores, and increased GnRH mRNA levels in

the hypothalamus (Klein et al., 1994; Sokol, 1987). Thus, these results indicate that Pb likely interferes with the male HPA axis, contributing to its reproductive toxicity.

In summary, recent epidemiologic studies have demonstrated an inverse relationship of Pb on pubertal development among boys at low blood Pb levels (mean and/or median blood Pb levels of 3.0 to 9.5 µg/dL). These studies were mostly cross-sectional, but associations were observed between blood Pb levels and delayed puberty in a longitudinal study as well (Williams et al., 2010). The larger studies controlled for some potential confounders, with a few studies at least considering the inclusion of dietary factors, which may be an important confounder, especially in populations with high prevalence of malnutrition. Some populations, such as the Russian boys cohorts, had potential exposures to dioxins and polychlorinated biphenyls, but these were not considered in the analyses with blood Pb levels. No recent toxicological studies were found that addressed the effect of Pb on male sexual development and maturation; however, the 2006 Pb AQCD (U.S. EPA, 2006b) supported earlier findings that Pb exposure may result in delayed onset of male puberty and altered reproductive function later in life in experimental animals.

4.8.1.3 Effects on Postnatal Stature and Body Weight

Findings from previous toxicological studies of rodents and primates have demonstrated Pb induced impairment of postnatal growth (U.S. EPA, 2006b). Little epidemiologic evidence was available in the 2006 Pb AQCD on postnatal growth. Several recent epidemiologic studies examining the association of various biomarkers of Pb exposure with stature and body weight have been conducted and the evidence reported is mixed. One new toxicology study reports effects on body weight.

Table 4-39 Summary of recent epidemiologic studies of associations between Pb biomarker levels and postnatal growth.

Reference[a]	Study Population Study Location Study Years	Methodological Details	Pb Biomarker Data Mean (SD)	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Afeiche et al. (2011)	n=523 boys n=477 girls Mexico City, Mexico Children born between 1994 and 2005	Longitudinal cohort using varying coefficient models with random effects	Maternal bone Pb 1 month postpartum Patella: 10.4 (11.8) µg/g	Change in weight at 5 years of age (g) per 1 SD increase in maternal bone Pb (95% CI): Girls: -171.6 (-275.2, -68.0) Boys: -35.0 (-132.4, 62.3)	Cohort, maternal age, calf circumference, height, education, number of pregnancies, breast feeding for 6 months, Ca^{2+} treatment, child's gestational age at birth, height, repeated measures of concurrent child blood Pb

Table 4-39 (Continued): Summary of recent epidemiologic studies of associations between Pb biomarker levels and postnatal growth.

Reference[a]	Study Population Study Location Study Years	Methodological Details	Pb Biomarker Data Mean (SD)	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Schell et al. (2009)	N=244 Albany, New York 1986-1992, and 1992-1998	Longitudinal cohort study using multivariate regression	Maternal blood Pb During second trimester: 2.8 (2.6) µg/dL During third trimester: 2.6 (2.2) µg/dL At delivery: 2.8 (2.4) µg/dL Infant blood Pb At delivery: 2.3 (2.7) µg/dL At 6 months: 3.2 (3.3) µg/dL At 12 months: 6.3 (4.8) µg/dL	β (p-value) for maternal second trimester Pb **Length for age (z-score):** 6 month: 0.149 (0.05) 12 month: 0.073 (0.38) **Weight for age (z-score):** 6 month: 0.013 (0.89) 12 month: 0.124 (0.25) **Weight for length (z-score):** 6 month: -0.158 (0.16) 12 month: 0.084 (0.45) **Head circumference for age (z-score):** 6 month: -0.242 (0.01) 12 month: -0.220 (0.05) Upper arm circumference for age (z-score): 12 month: -0.132 (0.25) Note: When examining second trimester maternal Pb ≥ 3 µg/dL, associations were observed for 6 mo weight for age, 6 mo weight for length, 6 and 12 mo head circumference, and 12 mo upper arm circumference for age	Infant sex, infant birth weight, infant nutrition, maternal age, marital status, employment, race, height, parity, second trimester smoking, and education.

Table 4-39 (Continued): Summary of recent epidemiologic studies of associations between Pb biomarker levels and postnatal growth.

Reference[a]	Study Population Study Location Study Years	Methodological Details	Pb Biomarker Data Mean (SD)	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Lamb et al. (2008)	N=309 mother child pairs Kosovo, Yugoslavia 1985-1986	Longitudinal cohort study using linear regression	Maternal blood Pb measured mid-pregnancy Pristina: 5.60 (1.99) µg/dL Mitrovica: 20.56 (7.38) µg/dL	Regression coefficients (95% CI) relating maternal blood Pb: To Height (cm): **Pristina** 1 yr: -0.61 (-2.24, 1.03) 4 yr: 0.79 (-1.71, 3.29) 6.5 yr: 0.15 (-2.43, 2.74) 10 yr: -0.09 (-3.69, 3.52) **Mitrovica** 1 yr: -0.30 (-2.55, 1.96) 4 yr: -0.72 (-3.26, 1.82) 6.5 yr: -1.87 (-4.38, 0.64) 10 yr: -2.87 (-6.21, 0.47) To BMI (kg/m^2): **Pristina** 1 yr: 0.61 (-0.28, 1.50) 4 yr: 0.17 (-0.67, 1.00) 6.5 yr: 0.61 (-0.09, 1.30) 10 yr: -0.49 (-1.45, 0.46) **Mitrovica** 1 yr: 0.23 (-0.84, 1.30) 4 yr: 0.16 (-0.66, 0.98) 6.5 yr: -0.12 (-0.90, 0.66) 10 yr: 1.31 (-0.95, 3.57)	Infant sex, ethnicity, parity, maternal height or maternal BMI, maternal education, gestational age at delivery, gestational age at blood sample, HOME score

Table 4-39 (Continued): Summary of recent epidemiologic studies of associations between Pb biomarker levels and postnatal growth.

Reference[a]	Study Population Study Location Study Years	Methodological Details	Pb Biomarker Data Mean (SD)	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Ignasiak et al. (2006)	school children ages 7-15 yr n=463 boys n= 436 girls South-western Poland 1995 (Industrial area with Cu smelters and refineries)	Cross-sectional study using stepwise multiple regression analysis	Concurrent blood Pb 7.7 (3.5) µg/dL	Estimated decrement per 10 µg/dL increase in blood Pb (p-value) **Weight:** Boys: 2.8 kg (0.002) Girls: 3.5 kg (0.007) **Height:** Boys: 3.2 cm (0.10) Girls: 4.0 cm (0.001) **Trunk length:** Boys: 1.2 cm (0.02) Girls: 1.1 cm (0.0001) **Leg length:** Boys: 2.1 cm (0.002) Girls: 2.9 cm (0.0001) **Arm length:** Boys: 1.8 cm (0.0001) Girls: 1.9 cm (0.008)	Age, age^2, education
Hauser et al. (2008)	N=489 boys ages 8-9 yr Chapaevsk, Russia May 2003 – May 2005	Cross-sectional study using multiple linear regression	Concurrent blood Pb Median (25th-75th percentile) 3 (2-5) µg/dL	Regression coefficient (95% CI) **Height (cm):** -1.439 (-2.25, -0.63) **Weight (kg):** -0.761 (-1.54, 0.02) **BMI (kg/m^2):** -0.107 (-0.44, 0.23)	Birth weight, gestational age, age at exam

Table 4-39 (Continued): Summary of recent epidemiologic studies of associations between Pb biomarker levels and postnatal growth.

Reference[a]	Study Population Study Location Study Years	Methodological Details	Pb Biomarker Data Mean (SD)	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Little et al. (2009)	n=196 (1980s) n=169 (2002) ages 2-12 yr Dallas, Texas 1980-1989 and 2002	Cross-sectional study using MANOVA, MANCOVA, and regression models	Concurrent blood Pb 1980s: 23.6 (1.3 SE) µg/dL 2002: 1.6 (0.2 SE) µg/dL	Changes in mean scaled measure per 10 µg/dL Pb increase (95%CI): **Height (cm):** -2.1 (-1.9, -2.3) **Weight (kg):** -1.9 (-1.7, -2.1) **BMI (kg/m^2):** -0.5 (-0.4, -0.7)	Age, age^2, sex and cohort effect
Min et al. (2008b)	n=62 boys n= 46 girls ages 5-13 yr Seoul, South Korea Date(s) not specified	Cross-sectional study using multiple linear regression	Concurrent blood Pb 2.4 (0.7) µg/dL	Linear model estimate (SE; p-value) **Height (cm):** -1.449 (0.639; p=0.026) Total arm length (cm): -1.804 (0.702; p=0.012) **Body weight (kg):** -0.646 (0.718; p=0.370) **BMI (kg/m^2):** -0.006 (0.272; p=0.982)	Age, sex, and father's education

Table 4-39 (Continued): Summary of recent epidemiologic studies of associations between Pb biomarker levels and postnatal growth.

Reference[a]	Study Population Study Location Study Years	Methodological Details	Pb Biomarker Data Mean (SD)	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Sanna and Vallascas (2011)	N = 825 children ages 11-14 yr Sardinia, Italy Data collected in 1998, 2002 and 2007	Cross-sectional study using multiple regression analysis	Pb in hair 1998: 5.84 (6.56) µg/g 2002: 1.49 (1.72) µg/g 2007: 0.78 (0.93) µg/g	β log Pb (p-value) **Height (mm)** 1998: -0.121 (0.0021) 2002: -0.115 (0.0349) 2007: 0.011 (0.8665) **Sitting Height (mm)** 1998: -0.117 (0.0017) 2002: -0.036 (0.5149) 2007: 0.028 (0.6633) **Estimated Lower Limb Length (mm)** 1998: -0.103 (0.0209) 2002: -0.164 (0.0057) 2007: -0.008 (0.9058)	Age, sex
Zailina et al. (2008)	N = 269 children ages 6.5-8.5 yr n=169 urban n=100 industrial Kuala Lumpur, Malaysia	Cross-sectional study using correlations	Concurrent blood Pb Industrial: 3.75 µg/dL Urban: 3.56 µg/dL	Correlation with blood Pb (p-value): Height for age: Urban: -0.095 (0.219) Industrial: -0.037 (0.716) **Weight for age:** Urban: 0.019 (0.806) Industrial: -0.063 (0.535) **Weight for height:** Urban: 0.136 (0.079) Industrial: -0.069 (0.493) **Left arm circumference:** Urban: 0.041 (0.595) Industrial: -0.055 (0.587)	None

Table 4-39 (Continued): Summary of recent epidemiologic studies of associations between Pb biomarker levels and postnatal growth.

Reference[a]	Study Population Study Location Study Years	Methodological Details	Pb Biomarker Data Mean (SD)	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Tomoum et al. (2010)	n=41 boys and girls ages 10-13 yr Cairo, Egypt Jan-Jun 2007	Cross-sectional study using t-test or Mann-Whitney U-test	Concurrent blood Pb 9.46 (3.08) µg/dL	Percentage of the median (SD): Pb<10 µg/dL **Weight:** Boys: 127.56 (16.26) Girls: 114.8 (10.8) **Height:** Boys: 98.06 (3.19) Girls: 96.75 (2.91) Pb≥ 10 µg/dL **Weight:** Boys: 122.0 (16.71) Girls: 123.11 (12.52) **Height:** Boys: 99.5 (5.04) Girls: 100.33 (4.53) p-value for all comparisons >0.05	None
Olivero-Verbel et al. (2007)	N=189 children ages 5-9 yr Cartagena, Columbia Jun-Aug 2004	Cross-sectional study using Spearman correlations	Concurrent blood Pb 5.49 (0.23) µg/dL	Spearman correlation coefficient (p-value) between blood Pb and Body size (cm): -0.224 (0.002) **Weight (kg):** -0.126 (0.087) *no significance in partial correlation between blood Pb and size when controlled for age: -0.096 (0.189)	None

Table 4-39 (Continued): Summary of recent epidemiologic studies of associations between Pb biomarker levels and postnatal growth.

Reference[a]	Study Population Study Location Study Years	Methodological Details	Pb Biomarker Data Mean (SD)	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Liu et al. (2011b)	N=303 ages 3-7 yr Guiyu, China Chendian, China Jan-Feb 2008	Cross-sectional study using sample t-tests	Concurrent blood Pb Guiyu: 13.2 (4.0-48.5) µg/dL Chendian: 8.2 (0-21.3) µg/dL Median (range)	Mean chest circumference among girls (cm): **<10 µg/dL**: 50.31 +/- 3.22 cm **≥ 10 µg/dL:** 49.03 +/- 2.27 cm (p-value <0.05) Mean chest circumference among children >6 years old (cm): **<10 µg/dL:** 51.70 +/- 3.35 cm **≥ 10 µg/dL:** 52.87 +/- 2.49 cm (p-value <0.05) Mean head circumference among children >6 years old (cm): **<10 µg/dL:** 48.71 +/- 1.66 cm **≥ 10 µg/dL:** 50.04 +/- 1.29 cm (p-value <0.01)	None
Mahram et al. (2007)	n=42 boys n= 39 girls n=45 exposed n=36 controls ages 7-11 yr Zanjan province, Iran Date(s) not specified	Cross-sectional study using t-tests	Concurrent blood Pb Area with Pb mining: 37.0 (24.7) µg/dL Area without Pb mining: 15.6 (13.4) µg/dL	Comparison of children living in areas with a Pb mine to children living in areas without a Pb mine: **Height (cm),** standardized for age: p-value 0.52 **Weight (kg),** standardized for age: p-value 0.8	None

[a]Studies are presented in order of first appearance in the text of this section, which is based on study design and methodological strength.

Results from recent epidemiologic studies of postnatal growth are summarized in Table 4-39. Longitudinal epidemiologic studies have had inconsistent findings regarding the association between Pb levels and post-natal growth. Afeiche et al. (2011) conducted a longitudinal study of children in Mexico City, born between 1994 and 2005. Maternal bone Pb during pregnancy was associated with a statistically significant decrease in weight at age 5 years in girls but not in boys. The findings were robust to additional adjustment for child's concurrent blood Pb level. A study in New York reported an inverse association between maternal blood Pb during the second trimester of pregnancy and various measures of growth, especially among those mothers with blood Pb levels of at least 3 µg/dL (Schell et al., 2009). These associations did not persist for those with maternal blood Pb levels less than 3 µg/dL. Among infants, age 6 month blood Pb levels were not associated with measures of growth at age 12 months. In comparisons of changes in blood Pb levels over time, high maternal blood Pb combined with low 12 month blood Pb among infants (indicating a decrease in blood Pb over time) resulted in the greatest growth, even compared to those with both low, or both high, maternal and infant blood Pb measures. In a prospective study of 309 mother-child pairs from Yugoslavia, the relationship between maternal blood Pb measured mid-pregnancy and attained height in children was investigated in those living in a highly exposed town with a smelter and battery plant and those living in a relatively lower exposed town (Lamb et al., 2008). In multivariate adjusted regression models, neither attained height (at birth, 1, 4, 6.6, or 10 years of age) nor rate of height change per month (at birth-1 year, 1-4 years, 4-6.5 years, 6.5-10 years of age) was associated in a consistent direction with maternal pregnancy blood Pb levels in either the industrial or less exposed town. Weight was also not associated with maternal blood Pb in this study.

Multiple cross-sectional studies reported an association between Pb levels and impaired growth. Ignasiak et al. (2006) studied school children aged 7-15 years living close to Cu smelters and refineries in Poland to assess the impact of Pb exposure on their growth status. There was a statistically significant linear relationship between concurrent blood Pb and reduced weight, height, trunk, leg and arm lengths. This decrease in height was more influenced by decreases in leg length than trunk length. These results also indicated that there was attenuation in osteoblast activity associated with higher blood Pb levels, consistent with animal toxicological studies (Long et al., 1990). Hauser et al. (2008) investigated the relationship between blood Pb and height in boys living in Chapaevsk, Russia, an area contaminated with multiple pollutants including dioxins and metals. In a multivariate adjusted regression analysis, height significantly decreased with increasing blood Pb. Statistically nonsignificant decreases in weight and BMI were also observed. The association of blood Pb with height, weight, and BMI was examined among two cohorts of children living near Pb smelters in Texas (Little et al., 2009). The first cohort included children 2-12 years old in 1980 and the second cohort included children of the

same age in 2002 when blood Pb levels were substantially lower. Decreases in height, weight, and BMI with increasing blood Pb levels were observed among children in both cohorts and increases in height and weight were observed comparing children from the 2002 cohort to those from the 1980 cohort. In a study with Korean children, Min et al. (2008b) observed that height and total arm length decreased significantly with increasing blood Pb in multivariate adjusted regression models. A statistically nonsignificant decrease in body weight was observed with increasing blood Pb while no effect on BMI was reported. In a study of children in Sardinia Italy, Sanna and Vallascas (2011) measured Pb in hair at three points in time (1997, 2002, and 2007) and reported cross-sectional results from regression analyses for each of these time periods. Pb in hair decreased over time and significant associations of Pb in hair with height were observed only in earlier time periods when hair Pb levels were relatively high. However, Pb in hair samples is not a well-characterized biomarker (see Chapter 3 and Section 3.3.4.2).

Contrary to the results summarized above, several cross-sectional studies do not observe associations between blood Pb levels and impaired growth. In a study with a similar design, Zailina et al. (2008) studied the relationship of blood Pb and height in 7 year-old Malaysian school children comparing those attending two schools in an urban setting to those attending a school near an industrial area. After adjustment for age, no statistically significant associations between concurrent blood Pb and physical development were observed. Tomoum et al. (2010) investigated the association between blood Pb and height in pubertal children in Cairo, Egypt. Neither boys nor girls with concurrent blood Pb levels >10 µg/dL differed significantly in height or weight when compared to those with blood Pb <10 µg/dL. In a simple correlation analysis of children aged 5-9 years in Colombia, Olivero-Verbel et al. (2007) reported that concurrent blood Pb levels were negatively associated with body size ($r = -0.224$, $p < 0.002$). However, when a partial correlation analysis was performed controlling for age, the association between blood Pb and body size was no longer statistically significant. In a study of school children in China, chest and head circumference were found to differ between high (>10µg/dL) and low concurrent blood Pb level groups; however, the direction of the difference was not consistent (Liu et al., 2011b). Among girls, in comparison of those with high and low blood Pb levels, a reduction in head circumference was observed. Among children greater than 6 years of age, those with higher blood Pb levels were reported to have greater head and chest circumferences. In a study of children aged 7-11 years and living in an area of Iran with or without Pb smelters, age-standardized weight and height did not vary by study area (Mahram et al., 2007).

Evidence from previous toxicological studies has shown an association between gestational Pb exposure and impaired postnatal growth (U.S. EPA, 2006b). Recent toxicological studies report significant changes in postnatal or adult body weight after Pb

exposure during different developmental windows. Masso-Gonzalez and Antonio-Garcia (2009) found Pb-induced decreased body weights at weaning (PND21) in rat pups from dams exposed to Pb during pregnancy and lactation (drinking water, 300 mg/L). Blood Pb level in the control group was 1.43 µg/dL; in the Pb group it was 22.8 µg/dL. Dong et al. (2009) reported decreased body weight in adult Kunming mice after exposure to 6,000 ppm Pb acetate in drinking water for 8 weeks. In contrast, Leasure et al. (2008) reported a statistically significant inverse relationship between Pb dose and body weight for male mice exposed to lower (27 ppm), moderate (55 ppm) and higher levels (109 ppm) levels of Pb during gestation and lactation (dam drinking water, 2 weeks before mating, through gestation and to PND10) with those exposed to the lowest dose having the highest adult body weight among the overweight Pb-exposed animals. Male mice exposed to 27 and 109 ppm Pb concentrations during gestation were 26% and 13% heavier than were controls at 1 year of age, respectively. In this study, dams were administered 27 ppm (lower), 55 ppm (moderate), and 109 ppm (higher) Pb in drinking water beginning 2 weeks prior to mating and going through gestation and lactation to PND10, which resulted in respective blood Pb levels from 10 µg/dL or less in the lower-exposure offspring to 42 µg/dL in the higher-exposure offspring at PND10. Leasure et al. (2008) also exposed a separate group of mice to Pb only during the postnatal period (PND0-PND21, lactation only exposure), and mice exposed to the same aforementioned lower or higher dose of Pb did not exhibit a difference in body weight when compared to control offspring. Wang et al. (2009e) observed a statistically significant decrease in fetal body weight and body length of Wistar rats at GD20 after maternal exposure to 250 ppm Pb acetate during gestation days 1-10, 11-20, or 1-20. Also, associations were reported between elevated maternal blood Pb levels (0.6, 1.3, or 1.74 µM, respectively or ~12.4, 26.9, or 36.0 µg/dL, respectively) compared to control (0.04 µM or ~0.83 µg/dL) and decreased pup body length and placental weight in Wistar rats at GD20. The greatest decrease in fetal body weight and length was observed in the group exposed to Pb during gestation days 1-20 followed by the group exposed to Pb during gestation days 11-20. Teijón et al. (2006) observed reductions in birth weight of litters administered 200 ppm or 400 ppm Pb acetate in drinking water (Wistar rats, Pb to dams from GD1 through lactation, and to 1 and 3 months postweaning to pups), but found that this effect did not persist in the postnatal growth of the rats.

Notably, previous toxicological studies observed reductions in postnatal weight as well as birth weight after exposure to Pb, albeit often at higher concentrations of Pb exposure. Ronis et al. (2001; 1998a; 1998b; 1996) have published a series of papers reporting on experiments exposing rats to Pb over different developmental windows, showing associations between Pb exposure and deficits in growth. Sprague-Dawley rats with lifetime Pb exposure to 6,000 ppm Pb acetate in drinking water (gestational-termination of experiment Pb exposure, maximum blood Pb of 316 µg/dL in males and 264 µg/dL in

females) had sex-independent pre-pubertal growth suppression, male-specific suppression of pubertal growth and loss of growth effects postnatally but still maintained an overall decreased body size out to PND60 due to earlier deficits. In a follow up study using the same exposure duration with a dose of 4,500 ppm Pb acetate (resulting in blood Pb of 263 µg/dL at PND85) yielded the same results (Ronis et al., 1996) with mechanistic insight showing decrements in insulin-like growth factor 1 (IGF1) accompanying the decreases in growth rates.

In summary, the body of toxicological literature on postnatal growth with Pb exposure indicates that Pb exposure can induce decrements in both height/body length and BW that may be persistent and differ by sex. However, findings from epidemiologic studies of postnatal growth are not consistent. Many of these studies were limited by their cross-sectional design. A few studies used longitudinal cohorts and controlled for multiple potential confounders, such as age and parity, but the results of these studies are inconsistent. Animal toxicology studies give insight to mechanistic changes that may contribute to this Pb-induced decrement and to the windows of exposure that may contribute greatest to these decrements.

4.8.1.4 Toxicological Studies of Other Developmental Effects

Developmental Effects on Blood and Liver

The 1986 and 2006 Pb AQCDs [(U.S. EPA, 1986b) and (U.S. EPA, 2006b)] reported studies that suggest Pb may alter hematopoietic and hepatic function during development. Some recent studies provide evidence that support these findings; however recent results are not consistent among the studies.

Massó et al. (2007) reported a decrease in liver weights of pups born to dams that consumed 300 mg/L Pb in drinking water during gestation and lactation. They also reported an increase in the number of erythrocytes; however the erythrocyte size was diminished by 62%. Pb produced microcytic anemia as evidenced by decreased hemoglobin content and hematocrit values without changes in mean corpuscular hemoglobin (MCH) concentration. Alkaline phosphatase (ALP) activity, CAT activity, or thiobarbituric acid reactive substances (TBARS) production did not change in pups at PND 0, but increased statistically significantly by PND21 indicating reactive oxygen generation. No change in acid phosphatase (ACP) activity was observed in the livers of pups at PND0 or PND21.

Massó-González and Antonia-García (2009) reported normochromic and microcytic anemia and a significant decrease in hematocrit values and blood δ-aminolevulinic acid dehydratase (ALAD) activity (90% reduction) in pups from dams administered 300 mg/L Pb acetate in drinking water during gestation. The authors also reported that erythrocyte osmotic fragility was four times greater in Pb-exposed pups than in control pups. Massó-González and Antonia-García (2009) reported increases in TBARS and CAT activity in the liver after Pb exposure. Intoxication with Pb also resulted in decreased liver protein concentrations and Mn-dependent SOD activity. Abnormalities in liver function were further exemplified by increases in liver concentrations of ALP and ACP.

Teijón et al. (2006) observed that gestational exposure to Pb caused a decrease in erythrocytes, hemoglobin, and MCH at weaning; however, by 1 and 3 months postweaning, these parameters had returned to normal values. The authors observed a slight increase in serum ALP, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) levels after Pb exposure in the absence of liver histological changes.

Pb-induced effects on SOD activity in the liver of fetuses after Pb intoxication was supported by a study by Uzbekov et al. (2007). The authors reported an initial increase in SOD activity in livers of pups exposed to 0.3 mg/L and 3.0 mg/L Pb nitrate in drinking water during gestation for 1 month (mean daily consumption 27 µg/kg). In contrast, long-term exposure (5 months) to the same concentrations of Pb nitrate concentration during gestation resulted in decreased hepatic SOD activity.

Effects on hepatic Phase I and Phase II enzymes after early developmental exposure of offspring to Pb during gestation and lactation was evaluated by Pillai et al. (2009). In the study, pregnant Charles Foster rats were administered 0.05 mg/kg body weight Pb subcutaneously throughout gestation until PND21. Pups were evaluated on PND56. Results of the study show that Phase I xenobiotic-metabolizing enzymes (NADPH- and NADH cytochrome c reductase) and Phase II xenobiotic- and steroid-metabolizing enzymes (δ-glutamyl transpeptidase, UDPGT, glutathione-s-transferase, and 17β-hydroxysteroid oxidoreductase) were reduced in both male and female pups by PND56. Only inhibition in glutathione-s-transferase and 17β-hydroxysteroid oxidoreductase activities demonstrated a sex-specific pattern (glutathione-s-transferase inhibition in males; 17β-hydroxysteroid oxidoreductase inhibition greater in females). Observed Pb-induced histological changes included massive fatty degeneration in hepatocytes, large vacuoles in cytoplasm, appearance of pyknotic nuclei, and infiltration of lymphocytes in the liver. Activities of antioxidant enzymes (SOD, CAT, glutathione peroxidase, and glutathione reductase) were also reduced after Pb intoxication. Alterations in biochemical parameters included decreased DNA, RNA, and cholesterol

content, although it was not clear whether these changes were related to genetic expression of xenobiotic-metabolizing enzymes or changes in steroid hormone homeostasis.

Developmental Effects on Skin

The 2006 Pb AQCD (U.S. EPA, 2006b) reported a study that demonstrated Pb-induced abnormalities in skin development. No current studies were identified that addressed Pb-induced skin alterations.

Developmental Effects on the Retina

The 2006 Pb AQCD (U.S. EPA, 2006b) concluded that Pb exposure during early postnatal development (resulting in blood Pb levels ~20 µg/dL) impaired retinal development in female Long-Evans hooded rats. A more recent study (Fox et al., 2008) exposed female Long-Evans hooded rats to lower (27 ppm), moderate (55 ppm), and higher (109 ppm) levels of Pb acetate in drinking water beginning 2 weeks before mating, throughout gestation, and until PND10. Blood Pb levels measured in these pups on postnatal days 0-10 were 10-12 µg/dL (lower), 21-24 µg/dL (moderate), and 40-46 µg/dL (higher). Results of the study demonstrated supernormal persistent rod photoreceptor-mediated (scotopic) electroretinograms (ERGs) [(Fox et al., 2008), and Table 4-13] in adult rats similar to ERG findings in male and female children in association with maternal first trimester blood Pb levels 10.5-32 µg/dL [(Rothenberg et al., 2002b), and Table 4-13]. In rats, lower and moderate levels of Pb increased neurogenesis of rod photoreceptors and rod bipolar cells without affecting Müller glial cells and statistically significantly increased the number of rods in central and peripheral retina. Higher-level Pb exposure (109 ppm) statistically significantly decreased the number of rods in central and peripheral retina. Pb exposure induced concentration-dependent decreases in adult rat retinal dopamine synthesis and utilization/release.

Developmental Effects on Teeth

Pb has been associated with multiple health effects including dental caries; however, there is very limited information available on the temporal and spatial incorporation of Pb in dental tissue (Arora et al., 2005). Arora et al. (2005) demonstrated that Wistar rat pups exposed to Pb during gestation and lactation (40 mg/L of Pb nitrate in drinking water of pregnant dams) had higher concentrations of Pb on the surface of enamel and in the dentine immediately adjacent to the pulp.

4.8.2 Effects on Birth Outcomes

The 2006 Pb AQCD reported on multiple studies of adverse birth outcomes such as, fetal mortality, birth defects, preterm birth, and low birth weight/fetal growth (U.S. EPA, 2006b). The toxicological studies reviewed in the 2006 Pb AQCD concluded that Pb exposure can increase fetal mortality and produce sublethal effects, smaller litters, and fewer implantation sites. Epidemiologic studies using occupational histories reported the possibility of small associations between increased Pb exposure and birth defects, and toxicological studies demonstrated associations between exposure to high doses of Pb and increased incidences of teratogenic effects in experimental animals. Epidemiologic studies on preterm birth and low birth weight/fetal growth included in the 2006 Pb AQCD reported inconsistent findings. Evidence from previous toxicological studies has shown an association between gestational Pb exposure and reduced birth weight and decreased litter size or number of pups. Continued research on adverse birth outcomes is described in the sections that follow. The studies presented in the following text and tables are grouped by study design and methodological strength.

4.8.2.1 Infant Mortality and Embryogenesis

No recent epidemiologic or toxicological studies have reported on the relationship between Pb levels and infant mortality. The 2006 Pb AQCD (U.S. EPA, 2006b) concluded that Pb exposure can increase fetal mortality and produce sublethal effects (disrupt growth and development) in offspring of Pb exposed dams at concentrations that do not result in clinical toxicity to the dams by disrupting implantation and pregnancy, particularly at the blastocyst stage of development. In rodent studies gestational exposure to Pb (blood Pb levels 32 to >70 μg/dL) resulted in smaller litters and fewer implantation sites and in non-human primates pre- and perinatal mortality was reported in squirrel monkeys exposed to Pb (mean dam blood Pb level of 54 μg/dL) in the last two-thirds of gestation (U.S. EPA, 2006b). There is substantial evidence to show that there is no apparent maternal-fetal barrier to Pb and it can easily cross the placenta and accumulate in fetal tissue during gestation (Pillai et al., 2009; Wang et al., 2009e; Uzbekov et al., 2007).

4.8.2.2 Birth Defects

The 2006 Pb AQCD (U.S. EPA, 2006b) reported the possibility of small associations between high Pb exposure and birth defects with many of the epidemiologic studies using occupational histories. Among the studies included in the 2006 Pb AQCD, a couple

reported associations between parental exposure to Pb and neural tube defects (Irgens et al., 1998; Bound et al., 1997). Recent studies also examined indicators of Pb exposure and neural tube defects (Table 4-40). No other recent epidemiologic studies of Pb exposure and birth defects were identified in the literature. No recent toxicological studies were found that investigated Pb-induced changes in morphology, teratology effects, or skeletal malformations of developing fetuses as a result of maternal Pb exposure; however, in the 2006 Pb AQCD toxicological studies demonstrated associations between exposure to high doses of Pb and increased incidences of teratogenic effect in experimental animals.

Table 4-40 Summary of recent epidemiologic studies of associations between Pb exposure indicators and neural tube defects.

Reference[a]	Study Population Study Location Study Years	Methodological Details	Data for Pb Biomarker or Exposure Measures Mean (SD)	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Zeyrek et al. (2009)	Infants with gestational age of at least 20 weeks N_{NTD}=74 $N_{controls}$=70 Turkey Years not specified	Case-control study using Student's t-test and Mann-Whitney U-test	Maternal and umbilical cord blood Pb taken 0.5h after birth Cases: Maternal: 15.5 (15.0) µg/dL Umbilical cord: 18.2 (17.8) µg/dL Controls: Maternal: 12.5 (12.7) Umbilical cord: 16.5 (16.1)	P-values for differences of Student's t-test or Mann-Whitney U-test (dependent on distribution) were 0.35 for maternal blood Pb and 0.63 for umbilical cord blood Pb	None
Brender et al. (2006)	Infants of Mexican-American women N_{NTD}=184 $N_{controls}$=225 Texas 1995-2000	Case-control study using logistic regression	Maternal blood Pb taken 5-6 weeks post-partum Cases: 2.4 (1.9) µg/dL Controls: 2.5 (1.6) µg/dL	OR (95% CI): Blood Pb<6.0 µg/dL: 1.0 (Ref) Blood Pb≥ 6.0 µg/dL: 1.5 (0.6, 4.3)	Inclusion of breast feeding in the model changed the OR (95% CI) to 3.8 (0.8, 19.5)
Huang et al. (2011b)	Live and still births of women living in the study area (villages in the Lvliang region of Shanxi province) N=112 villages China 2002-2004	Ecologic	2 soil samples from each village 56.14 µg/g (11.43 µg/g)	N/A	

[a]Studies are presented in order of first appearance in the text of this section, which is based on study design and methodological strength.

Among the recent epidemiologic studies (described in Table 4-40), a study of women in Turkey detected no difference between the blood Pb of mothers or the umbilical cord blood Pb of the newborns for healthy infants compared with infants with neural tube defects (cases of spina bifida occulta were excluded, but other forms of spina bifida were

included) (Zeyrek et al., 2009). Brender et al. (2006) performed a study of Mexican-American women living in Texas. Measurements were taken 5-6 weeks postpartum, which is a limitation of this study because the blood Pb levels may be different from those during the developmental period of gestation. The OR comparing women with at least 6 µg/dL blood Pb to those with less than 6 µg/dL blood Pb was 1.5 (95% CI: 0.6, 4.3). This increased after adjusting for breast feeding, although this variable was not a confounder because it cannot be associated with neural tube defects. For these women, neither occupational exposure to Pb nor proximity of residence to a facility with Pb air emissions at the time of conception was associated with increased odds of neural tube defects. A study with an ecologic design was performed in China and did not use individual-level biomarkers to determine Pb levels (Huang et al., 2011b). A positive association between Pb levels in soil samples and neural tube defects was reported. Exposure to multiple other trace elements also demonstrated a positive association but no control for co-exposures was included in the models for Pb.

In summary, previous studies included in the 2006 Pb AQCD observed associations between Pb and neural tube defects but were based on Pb was measured in drinking water (Bound et al., 1997) and estimated from occupational reports (Irgens et al., 1998). A recent ecologic study reported an association between Pb in the soil and neural tube defects but was also limited by its lack of biological samples, as well as a lack of individual-level data and the prevalence of several other metals (Huang et al., 2011b). Other recent epidemiologic studies of maternal blood Pb levels and neural tube defects observed no statistically significant associations (Zeyrek et al., 2009; Brender et al., 2006). These studies also have limitations, including the timing of Pb measurements and lack of control for potential confounders.

4.8.2.3 Preterm Birth

Epidemiologic studies on preterm birth included in the 2006 Pb AQCD (U.S. EPA, 2006b) reported inconsistent findings regarding the relationship between Pb and gestational age. Recent studies examined this potential association, and again mixed results were reported (Table 4-41). Of these studies, the ones that categorized births as preterm or term all defined preterm birth as less than 37 weeks of gestation. One limitation to note for these studies is that if Pb affects spontaneous abortion and length of gestation via a similar pathway, then the studies that only collect data at delivery and not at earlier stages of pregnancy would be biased toward the null.

Table 4-41 Summary of recent epidemiologic studies of associations between Pb exposure indicators and preterm birth.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Data for Pb Biomarker or Exposure Indicators	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Jelliffe-Pawlowski et al. (2006)	Preterm birth (< 37 completed week)	Singleton births to non-smoking mothers with blood Pb measures during pregnancy from either the California Childhood Lead Poisoning Prevention Branch or the California Occupational Lead Poisoning Prevention Program $N_{preterm\ birth}$=30 $N_{term\ birth}$=232 California 1995-2002	Longitudinal cohort study using logistic regression	Maximum maternal blood Pb during pregnancy ≥ 10 µg/dL: 30.9%	Odd Ratios: ≤ 5 µg/dL: 1.00 (Ref) 6-9 µg/dL: 0.8 (0.1, 6.4) 10-19 µg/dL: 1.1 (0.2, 5.2) 20-39 µg/dL: 4.5 (1.8, 10.9) ≥ 40 µg/dL: 4.7 (1.1, 19.9) <10 µg/dL: 1.00 (Ref) ≥ 10 µg/dL: 3.2 (1.2, 7.4)	In the 10 µg/dL dichotomized model: race, insurance, maternal age, parity, infant sex, low birthweight

Table 4-41 (Continued): Summary of recent epidemiologic studies of associations between Pb exposure indicators and preterm birth.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Data for Pb Biomarker or Exposure Indicators	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Vigeh et al. (2011)	Preterm birth (20-37 week)	Singleton births from non-smoking, non-obese mothers aged 16-35 yr and referred for prenatal care during the 8th-12th week of gestation $N_{preterm\ birth}$=44 $N_{term\ birth}$=304 Tehran, Iran 2006	Longitudinal cohort study using logistic regression	Maternal blood Pb at 8-12 weeks gestation Mean (SD) 3.8 (2.0) Preterm birth: 4.52 (1.63) Term birth: 3.72 (2.03) p-value for difference: <0.05	OR (95% CI): 1.41 (1.08, 1.84) (unit not given, assume per 1 µg/dL)	Age, infant sex, education, passive smoking exposure, pregnancy weight gain, parity, hematocrit, type of delivery

Table 4-41 (Continued): Summary of recent epidemiologic studies of associations between Pb exposure indicators and preterm birth.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Data for Pb Biomarker or Exposure Indicators	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Cantonwine et al. (2010a)	Preterm birth (<37week), Gestational age	Births to mothers with at least 1 blood Pb measurement during pregnancy and no chronic diseases requiring medication $N_{preterm\ birth}$=22 $N_{term\ birth}$=213 Mexico City 1997-1999	Longitudinal cohort study using linear regression	Maternal blood Pb during pregnancy Mean (SD) Blood Pb Visit at <20 weeks pregnant: 7.2 (5.2) Visit at 20-28 weeks pregnant: 6.3 (4.3) Visit at >28 weeks pregnant: 6.8 (4.5) Plasma Pb Visit at <20 weeks pregnant: 0.17 (0.16) Visit at 20-28 weeks pregnant: 0.13 (0.10) Visit at >28 weeks pregnant: 0.16 (0.26)	β (95% CI) per SD increase in centered log-Pb concentration Blood Pb Visit at <20 weeks: -2.76 (-5.21, -0.31) Visit at 20-28 weeks: -1.77 (-3.39, -0.15) Visit at >28 weeks: -0.47 (-1.78, 0.84) Average: -1.49 (-3.63, 0.64) Plasma Pb Visit at <20weeks: -2.38 (-4.97, 0.21) Visit at 20-28 weeks: -1.34 (-2.98, 0.29) Visit at >28 weeks: -1.28 (-2.63, 0.06) Average: -0.28 (-2.81, 2.25) Plasma-to-blood Pb ratio Visit at <20 weeks: -3.23 (-6.01, -0.44) Visit at 20-28 weeks: -1.41 (-3.10, 0.29) Visit at >28 weeks: -1.30 (-2.67, 0.07) Average: -1.27 (-3.89, 1.35) Cord blood Pb -0.68 (-2.37, 1.00)	Infant sex, maternal age, maternal education, history of adverse birth outcomes, cigarette smoking, parity

Table 4-41 (Continued): Summary of recent epidemiologic studies of associations between Pb exposure indicators and preterm birth.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Data for Pb Biomarker or Exposure Indicators	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Zhu et al. (2010)	Preterm birth (<37 completed week)	Singleton births to mothers aged 15-49 yr with blood Pb measures before or on the date of delivery and blood Pb measuring <10 µg/dL $N_{preterm\ birth}$=3519 $N_{term\ birth}$=39,769 New York 2003-2005	Retrospective cohort study using logistic regression with fractional polynomials	Maternal blood Pb Mean: 2.1	Odd Ratios: ≤ 1.0 µg/dL: 1.00 (Ref) 1.1-2.0 µg/dL: 1.03 (0.93, 1.13) 2.1-3.0 µg/dL: 1.01 (0.92, 1.10) 3.1-9.9 µg/dL: 1.04 (0.89, 1.22)	Timing of Pb test, maternal age, race, Hispanic ethnicity, smoking status, drug abuse, marital status, special financial program participation, parity, infant sex

Table 4-41 (Continued): Summary of recent epidemiologic studies of associations between Pb exposure indicators and preterm birth.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Data for Pb Biomarker or Exposure Indicators	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Chen et al. (2006a)	Preterm birth (<37 week)	Infants born to at least one parent who was part of the Program to Reduce Exposure by Surveillance System – Blood Lead Levels cohort that monitored workers occupationally exposed to Pb $N_{preterm\ birth}$=74 $N_{term\ birth}$=1537 *738 births had maternal Pb information and 967 had paternal Pb information Taiwan 1993-1997	Occupational cohort study using regression models	Maternal blood Pb during pregnancy (or if that wasn't available, the 1 year prior to fertilization) and/or paternal blood Pb during spermatogenesis (the 64 days before fertilization, or if that wasn't available, the 1 year prior to spermatogenesis) Mean (SD) Maternal blood Pb: 10.1 (10.4) Paternal blood Pb: 12.9 (13.8)	Risk Ratios Maternal blood Pb <10 µg/dL: 1.00 (Ref) 10-19 µg/dL: 1.97 (0.92, 3.86) ≥ 20 µg/dL: 1.86 (0.68, 4.28) Paternal blood Pb <10 µg/dL: 1.00 (Ref) 10-19 µg/dL: 1.17 (0.53, 2.32) ≥ 20 µg/dL: 0.55 (0.19, 1.28)	Parental age, parental education, parity, infant sex
Patel and Prabhu (2009)	Gestational age	Consecutive births at the study hospital N=205 (mean gestational age 39 +/-2 weeks) Nagpur, India Years not specified	Cross-sectional study using linear regression	Umbilical cord blood Pb Mean (SD): 4.7 (12.1)	>5 µg/dL: mean gestational age 38 weeks ≤ 5 µg/dL: mean gestational age 39 weeks Linear regression: gestational age decreased 1 week with every 1 µg/dL increase in umbilical cord blood Pb (exact values and 95% CI: not given)	Not specified

Table 4-41 (Continued): Summary of recent epidemiologic studies of associations between Pb exposure indicators and preterm birth.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Data for Pb Biomarker or Exposure Indicators	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Jones et al. (2010)	Gestational Age: preterm (<37week), term (37-40 week), post-term (>40 week)	Singleton births ≥ 27 week gestation from mothers aged 16-45 yr living in the Shelby County area for at least 5 mo during pregnancy $N_{preterm\ birth}$=10 $N_{term\ birth}$=81 $N_{postterm\ birth}$=11 Tennessee 2006	Cross-sectional study comparing across geometric means (test not specified)	Umbilical cord blood Pb Mean (SD): 2.4 (4.3) Geometric mean: 1.3	Geometric Mean: Preterm birth: 1.4 Term birth: 1.2 Post-term birth: 1.3 p-value for difference: >0.10	None
Wells et al. (2011a)	Gestational age	Singleton births from the Baltimore Tracking Health Related to Environmental Exposures (THREE) study $N_{preterm\ birth}$=39 $N_{term\ birth}$=261 Baltimore, MD 2004-2005	Cross-sectional study using multivariable linear regression	Umbilical cord Pb Mean: 0.84 (95%: CI 0.72, 0.96) ≥ 5 µg/dL: 0.7%	Ratio for Pb concentration per 10 days of gestation: 0.99 (0.93, 1.06)	Maternal age, race, insurance, pre-pregnancy BMI, smoking status, gestational age, birthweight, average year of neighborhood home construction

Table 4-41 (Continued): Summary of recent epidemiologic studies of associations between Pb exposure indicators and preterm birth.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Data for Pb Biomarker or Exposure Indicators	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Berkowitz et al. (2006)	Preterm birth (<37week)	Singleton births with 28-45 week gestation $N_{preterm\ birth}$=7,843 $N_{term\ birth}$=162,035 Shoshone County, ID 1970-1981	Cohort study using logistic regression	Three time periods of two locations (unexposed and exposed/near smelter): pre-fire, "high-exposure period" (when a fire happened at the smelter and resulted in damages leading to high air Pb concentrations for 6 mo), and "post-fire" During the time of the fire, estimates of Pb in ambient air were as high as 30 µg/m^3	OR (90% CI) (unexposed location is referent group): Pre-fire: 0.93 (0.67, 1.28) High exposure: 0.68 (0.34, 1.35) Post-fire: 1.17 (0.95, 1.45)	Maternal age, infant sex, first birth, previous miscarriage or abortion
Orun et al. (2011)	Preterm birth (<37 week)	Births to mothers not occupationally exposed to toxic metals and living in a suburban but non-industrial area $N_{preterm\ birth}$=17 $N_{term\ birth}$=127 Turkey Years not specified	Cohort study using Mann-Whitney U-test	Breast milk 2 months post-partum Median: 20.6 µg/L >WHO limit (5 µg/L): 87%	Median Pb (IQR) >37 week: 20.6 (11.2, 29.2) µg/L ≤ 37 week: 20.4 (14.4, 27.9) µg/L p-value for Mann-Whitney U test: ≥ 0.05	None

[a]Studies are presented in order of first appearance in the text of this section, which is based on study design and methodological strength.

In a study taking place in California, women with information on blood Pb levels during pregnancy based on their participation in a surveillance program (reason for participation in the surveillance program was unknown but the authors speculate it was likely because of potential Pb exposure due to occupational or environmental exposures or a family member was identified as exposed to Pb) were matched with the birth certificates of their infants (Jelliffe-Pawlowski et al., 2006). Almost 70% of women had maximum blood Pb measurements <10 µg/dL with just over 60%being ≤5 µg/dL. Preterm birth was associated with higher blood Pb when comparing women with maximum pregnancy blood Pb levels ≥ 10 µg/dL to women with blood Pb levels <10 µg/dL in adjusted analyses. In analyses of maximum Pb levels further refined into additional categories, the odds of preterm birth were elevated among women with maximum blood Pb measurement ≥ 20 µg/dL compared with women with maximum blood Pb levels ≤ 5 µg/dL. A study in Iran also reported higher maternal blood Pb for preterm births than for term births (Vigeh et al., 2011). The women in this study had lower blood Pb levels than did those observed in the Jelliffe-Pawlowski et al. (2006) study. Higher maternal blood Pb level was associated with higher odds of preterm birth. Another study examining blood Pb and gestational age reported an inverse association between maternal blood Pb concentration and gestational age, especially for blood Pb levels early in pregnancy (Cantonwine et al., 2010a). However, in a study conducted in New York among women with blood Pb levels less than 10 µg/dL, no association was observed between blood Pb levels and preterm birth (Zhu et al., 2010). Similarly, a study of maternal and paternal blood Pb concentrations reported no association between maternal or paternal blood Pb levels and preterm birth (Chen et al., 2006a).

In another study, measurements of umbilical cord blood were taken after birth at a hospital in Nagpur, India (Patel and Prabhu, 2009). A sample of women had their blood Pb measured and among this sample, maternal blood Pb was correlated with the umbilical cord Pb levels. Mean gestational age differed between infants with >5 µg/dL cord blood Pb and infants with ≤ 5 µg/dL cord blood Pb. In a linear regression model, gestational age was found to decrease with increasing umbilical cord Pb levels. A study of women in Tennessee consisted primarily of African American women living in an urban setting (Jones et al., 2010). The mean level of umbilical cord blood Pb was slightly higher among infants born preterm but the difference was not statistically significant. Using umbilical cord blood Pb measures, a study reported no association between cord blood Pb levels and gestational age. The overwhelming majority (99.3%) of cord blood Pb concentrations among study participants was ≤ 5 µg/dL (Wells et al., 2011a).

A study of preterm birth included women living in two different residential areas over three different time periods (Berkowitz et al., 2006). One residential area had consistently lower exposures but the other had a 6-month period of high Pb emissions due to damage to emissions controls at a local Pb smelter (Pb measured in ambient air was up to 30 $\mu g/m^3$). Preterm birth rates were examined during three time periods: before, during, and after the time of higher Pb exposure. No association with preterm birth was observed between women living in the high exposure area compared to those in the low exposure area during any of the exposure time periods, but the number of preterm infants born during the period of higher exposure was small.

A study of breast milk in the second month postpartum reported no difference in breast milk Pb levels for those infants born preterm or term; however, a limitation of this study is that Pb levels were not measured until two months after the birth and may not reflect maternal blood Pb levels during pregnancy (Örün et al., 2011).

In summary, as in the 2006 Pb AQCD, (U.S. EPA, 2006b) recent epidemiologic studies report inconsistent findings for a relationship between indicators of Pb exposure and preterm birth. No patterns were apparent within type of exposure measurement or Pb level. Many of these studies are limited by the small number of preterm births and their cross-sectional design (i.e., studies of umbilical cord blood may not adequately characterized blood Pb levels earlier in pregnancy).A few studies utilized a longitudinal cohort design (Vigeh et al., 2011; Cantonwine et al., 2010a; Chen et al., 2006a; Jelliffe-Pawlowski et al., 2006), and although results among these studies were mixed some did report an association between maternal blood Pb during pregnancy and preterm birth. Most studies controlled for potentially important confounders, such as maternal age and smoking.

4.8.2.4 Low Birth Weight/Fetal Growth

The 2006 Pb AQCD reported inconsistent epidemiologic study results for the associations between Pb and birth weight/fetal growth but concluded that there could be a small effect of Pb exposure on birth weight and fetal growth (U.S. EPA, 2006b). Since then, multiple epidemiologic studies on the relationship between Pb exposure and birth weight and fetal growth have been published using various measures of exposure, such as air levels, umbilical cord blood, and maternal blood and bone. These studies are summarized in Table 4-42 (organized in the text and table by the type of Pb measurement and then by study design). Additionally, there have been a few recent toxicological studies evaluating the effect of Pb exposure during gestation on birth weight.

Table 4-42 Summary of recent epidemiologic studies of associations between Pb exposure indicators and low birth weight and fetal growth.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Data for Pb Biomarkers or other Exposure Indicators	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Gundacker et al. (2010)	Birth length, birth weight, head circumference	Infants of women recruited during their second trimester N=53 Vienna, Austria 2005	Cohort study using categorical regression	Median (IQR): Maternal blood Pb between week 34-38 of gestation 2.5 (1.8, 3.5) µg/dL Whole placentas 25.8 (21.0, 36.8) µg/kg Umbilical cord Pb shortly after birth 1.3 (0.8, 2.4) µg/dL Meconium samples in first five days after birth 15.5 (9.8, 27.9) µg/kg	Regression coefficients (units not given, assume results are per 10 µg/dL or 1 µg/kg) Birth length: Placenta Pb: 0.599 (SE: 0.154, p-value <0.001) Meconium Pb: -0.385 (SE 0.157, p-value 0.012) Birth weight: Placenta Pb: 0.658 (SE 0.136, p-value <0.001) Maternal blood Pb: -0.262 (SE 0.131, p-value 0.058)	Model for birth length: placenta Pb, gestational age, meconium Pb Model for birth weight: gestational age, placenta Pb, maternal blood Pb

Table 4-42 (Continued): Summary of recent epidemiologic studies of associations between Pb exposure indicators and low birth weight and fetal growth.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Data for Pb Biomarkers or other Exposure Indicators	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Zhu et al. (2010)	Birth weight, small for gestational age (birth weight for gestational age <10th percentile based on national birth weight by gestational week from weeks 25-42)	Singleton births to mothers aged 15-49 yr with blood Pb measures before or on the date of delivery and blood Pb measuring <10 µg/dL N_{LBW}=2,744 $N_{normal\ BW}$=40,544 N_{SGA}=4092 $N_{no\ SGA}$=39,084 New York 2003-2005	Retrospective cohort using linear regression with factional polynomials for birth weight and logistic regression with fractional polynomials for SGA	Maternal blood Pb before or at delivery Mean: 2.1 µg/dL	Difference in birthweight in grams: 0 µg/dL: Ref 1 µg/dL: -27.4 (-37.8, -17.1) 2 µg/dL: -38.8 (-53.4, -24.1) 3 µg/dL: -47.5 (-65.4, -29.6) 4 µg/dL: -54.8 (-75.5, -34.2) 5 µg/dL: -61.3 (-84.4, -38.2) 6 µg/dL: -67.2 (-92.5, -41.8) 7 µg/dL: -72.5 (-99.9, -45.2) 8 µg/dL: -77.6 (-106.8, -48.3) 9 µg/dL: -82.3 (-113.3, -51.2) 10 µg/dL: -86.7 (-119.4, -54.0) After exclusion of blood Pb <1 µg/dL, a 1 µg/dL increase in blood Pb was associated with a 7.0 g decrease in birthweight Odd Ratios for small for gestational age: ≤ 1.0 µg/dL: 1.00 (Ref) 1.1-2.0 µg/dL: 1.07 (0.98, 1.17) 2.1-3.0 µg/dL: 1.06 (0.98, 1.16) 3.1-9.9 µg/dL: 1.07 (0.93, 1.23)	Model for birth weight: Timing of Pb test, maternal age, race, Hispanic ethnicity, education, smoking status, alcohol use, drug abuse, marital status, financial assistance program participation, parity, infant sex Model for SGA: Timing of Pb test, maternal age, race, education, smoking status, drug abuse, marital status, financial assistance program participation, parity, infant sex

Table 4-42 (Continued): Summary of recent epidemiologic studies of associations between Pb exposure indicators and low birth weight and fetal growth.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Data for Pb Biomarkers or other Exposure Indicators	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Chen et al. (2006a)	Low birth weight (<2,500 g), small for gestational age (birth weight ≤ 10th percentile of sex- and gestational week weights for singletons in 1993-1996)	Infants born to at least one parent who was part of the Program to Reduce Exposure by Surveillance System – Blood Lead Levels cohort that monitored workers occupationally exposed to Pb N_{LBW}=72 $N_{normal\ BW}$=1,539 N_{SGA}=135 $N_{no\ SGA}$=1,476 *738 births had maternal Pb information and 967 had paternal Pb information Taiwan 1993-1997	Occupational cohort study using regression models	Mean (SD) Maternal blood Pb during pregnancy (or if that wasn't available, the 1 year prior to fertilization) 10.1 (10.4) Paternal blood Pb during spermatogenesis (the 64 days before fertilization, or if that wasn't available, the 1 year prior to spermatogenesis) 12.9 (13.8)	Risk Ratios Low birth weight Maternal blood Pb <10 µg/dL: 1.00 (Ref) 10-19 µg/dL: 2.22 (1.06, 4.26) ≥ 20 µg/dL: 1.83 (0.67, 4.20) Paternal blood Pb <10 µg/dL: 1.00 (Ref) 10-19 µg/dL: 0.83 (0.34, 1.75) ≥ 20µg/dL: 0.42 (0.12, 1.06) SGA Maternal blood Pb <10 µg/dL: 1.00 (Ref) 10-19 µg/dL: 1.62 (0.91, 2.75) ≥ 20µg/dL: 2.15 (1.15, 3.83) Paternal blood Pb <10 µg/dL: 1.00 (Ref) 10-19 µg/dL: 0.94 (0.49, 1.66) ≥ 20 µg/dL: 0.94 (0.51, 1.62)	Low birth weight models: parental age, parental education, infant sex, parity SGA models: parental age, parental education

Table 4-42 (Continued): Summary of recent epidemiologic studies of associations between Pb exposure indicators and low birth weight and fetal growth.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Data for Pb Biomarkers or other Exposure Indicators	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Jelliffe-Pawlowski et al. (2006)	Low birth weight (<2,500g) Small for gestational age (birth weight for gestational age <10th percentile of race- and gender- specific norms	Singleton births to non-smoking mothers with blood Pb measures during pregnancy from either the California Childhood Lead Poisoning Prevention Branch or the California Occupational Lead Poisoning Prevention Program and matched to birth records N_{LBW}=9 $N_{normal\ BW}$=253 N_{SGA}=17 $N_{no\ SGA}$=245 California 1995-2002	Longitudinal cohort study using logistic regression	Maximum maternal blood Pb during pregnancy ≥ 10 µg/dL: 30.9%	Odd Ratios: Low birth weight ≤ 5 µg/dL: 1.00 (Ref) 6-9 µg/dL: not calculated (n=0) 10-19 µg/dL: 2.7 (0.5, 14.8) 20-39 µg/dL: 1.5 (0.3, 7.7) ≥ 40 µg/dL: not calculated (n=0) <10 µg/dL: 1.00 (Ref) ≥ 10 µg/dL: 3.6 (0.3, 40.0) Small for gestational age ≤ 5 µg/dL: 1.00 (Ref) 6-9 µg/dL: not calculated (n=0) 10-19 µg/dL: 2.3 (0.6, 9.2) 20-39 µg/dL: 2.1 (0.7, 6.7) ≥ 40 µg/dL: not calculated (n=0) <10 µg/dL: 1.00 (Ref) ≥ 10 µg/dL: 4.2 (1.3, 13.9)	10 µg/dL model for birth weight: preterm birth, race, insurance, parity, maternal age, infant sex 10 µg/dL model for SGA: insurance, parity, maternal age

Table 4-42 (Continued): Summary of recent epidemiologic studies of associations between Pb exposure indicators and low birth weight and fetal growth.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Data for Pb Biomarkers or other Exposure Indicators	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Lamb et al. (2008)	Height and BMI at birth	Participants of the Yugoslavia Study of Environmental Lead Exposure, Pregnancy Outcomes, and Childhood Development N=292 Mitrovica and Pristina, Yugoslavia 1985-1986	Population-based prospective cohort study using linear regression	Mean (SD) Mid-pregnancy blood Pb Mitrovica: 20.56 (7.38) Pristina: 5.60 (1.99)	Regression Coefficients (95% CI) for 1 µg/dL increase in Pb: BMI (kg/m^2) Mitrovica: -0.18 (-0.69, 0.33) Pristina: -0.14 (-0.69, 0.42) Height (cm) Mitrovica: 0.43 (-0.83, 1.69) Pristina: 0.35 (-0.64, 1.34)	Infant sex, ethnicity, parity, maternal height or BMI, maternal education, gestational age at blood sample, gestational age at birth, quality of home environment
Iranpour et al. (2007)	Low birth weight (≤ 2,500g, >37weeks)	Full-term infants born at a hospital affiliated with Isfahan University N_{LBW}32 $N_{normal\ BW}$=34 Isfahan, Iran 2005	Cross-sectional study using t-tests and Spearman's correlations	Mean (SD) Maternal blood Pb within 12 h of delivery Cases: 12.5 (2.0) µg/dL Controls: 13.5 (2.7) µg/dL Umbilical cord Cases: 10.7 (1.7) µg/dL Controls: 11.3 (1.9) µg/dL	P-values for t-tests: Maternal blood Pb: 0.07 Umbilical cord blood Pb: 0.20 P-values for correlations: Maternal blood Pb and Birth weight: Low birth weight: 0.17 Normal birth weight: 0.3 Umbilical cord blood Pb and birth weight: Low birth weight: 0.84 Normal birth weight: 0.26	None

Table 4-42 (Continued): Summary of recent epidemiologic studies of associations between Pb exposure indicators and low birth weight and fetal growth.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Data for Pb Biomarkers or other Exposure Indicators	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Kordas et al. (2009)	Head circumference, birth weight, birth length	Infants of mothers receiving antenatal care at hospitals serving low-to-middle income populations (cross-sectional study of baseline info from Ca supplementation trial) N=474 Mexico City, Mexico 1994-1995	Cross-sectional study using linear regression	Maternal tibia Pb 1 month post-partum Mean (SD): 9.9 (9.8) µg/g Maternal blood Pb within 12 h of delivery ≥ 10 µg/dL: 27% Umbilical cord ≥ 10 µg/dL: 13.7%	Regression coefficients (SE) for each 1 µg/g increase in tibia Pb: Birth weight: -4.9 (1.8) Birth length: -0.02 (0.01) Head circumference: -0.01 (0.01; p-value <0.05) Women in 4th quartile tibia Pb (15.6-76.5 µg/g) delivered infants 140 g less than women with tibia Pb in the lowest quartile	Maternal age, pre-pregnancy BMI, maternal height, education, parity, marital status, ever smoker, postpartum calf circumference, gestational age, infant sex
Afeiche et al. (2011)	Birth weight	Term, singleton births, at least 2,500 grams enrolled in one of three birth cohorts recruited for other longitudinal studies N=1,000 Mexico City 1994-2005	Cross-sectional study using varying coefficient models with random effects	Mean (SD) measured 1 month postpartum Maternal patella Pb 10.4 (11.8) µg/g Maternal tibia Pb 8.7 (9.7) µg/g	β (95% CI) for 1 SD increase in maternal patella Pb Girls: -45.7 (-131.7, 40.2) Boys: 72.3 (-9.8, 154.4) No association for birth weight and tibia Pb among girls. A positive association was observed for tibia Pb and birth weight among boys (quantitative results not given).	Birth cohort, maternal age, maternal calf circumference, maternal height, education, parity, breast feeding, Ca^{2+} treatment group assignment, gestational age, height at birth, repeated concurrent child blood Pb measures

Table 4-42 (Continued): Summary of recent epidemiologic studies of associations between Pb exposure indicators and low birth weight and fetal growth.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Data for Pb Biomarkers or other Exposure Indicators	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Cantonwine et al. (2010b)	Birth weight	Infants who were part of a clinical trial to assess maternal Ca^{2+}-supplementation on bone Pb mobilization during lactation N=533 Mexico City 1994-1995	Cross-sectional study using linear regression	Umbilical cord blood Pb Means varied from 6.3 to 6.9 (categories created based on maternal HFE genotype) ≥ 10 µg/dL: 12.6% Maternal tibia and patella Pb one month after delivery	β (95% CI), in grams Umbilical cord blood Pb: -31.1 (-105.4, 43.3) per 1 µg/dL Maternal tibia Pb Overall: -4.4 (-7.9, -0.9) per 1 µg/g <1-4.1 µg/g: Ref 4.1-9.2 µg/g: 17.2 (-75.6, 110.1) 9.2-15.4 µg/g: -19.1 (-112.1, 73.9) 15.4-43.2 µg/g: -95.4 (-189.9, -0.8)	Maternal age, education, infant sex, maternal arm circumference, gestational age, smoking status during pregnancy, marital status, maternal hemoglobin first month postpartum, parity
Wells et al. (2011a)	Birth weight	Singleton births from the Baltimore Tracking Health Related to Environmental Exposures (THREE) study N_{LBW}=33 $N_{normal\ BW}$=267 Baltimore, MD 2004-2005	Cross-sectional study using multivariable linear regression	Umbilical cord Pb Mean (95% CI): 0.84 (0.72, 0.96) µg/dL ≥ 5 µg/dL: 0.7%	Ratio for Pb concentration per 100g birth weight: 1.01 (0.99, 1.02)	Maternal age, race, insurance, pre-pregnancy weight, smoking status, gestational length, birth weight, average year of neighborhood home construction

Table 4-42 (Continued): Summary of recent epidemiologic studies of associations between Pb exposure indicators and low birth weight and fetal growth.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Data for Pb Biomarkers or other Exposure Indicators	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Al-Saleh et al. (2008b)	Head circumference	Infants with a gestational age of at least 34 weeks born to healthy mothers aged 17-46 yr and non-occupationally exposed to Pb N=653 Saudi Arabia 2004	Cross-sectional study using linear regression	Umbilical cord blood Pb Mean (SD): 2.210 (1.691) µg/dL 75th percentile: 2.475 µg/dL >10 µg/dL: 1.23%	Regression models for those above the 75th percentile of cord blood Pb levels β (SE) per unit of log-transformed Pb -0.158 (0.718), p-value: 0.036	BMI, gestational age Considered but not included: prenatal supplements, location of residence
Atabek et al. (2007)	Birth weight, birth length, head circumference, mid-arm circumference	Term, singleton infants born to healthy mothers living in urban areas and assumed to have high Pb concentrations N=54 Turkey NS	Cross-sectional study using linear regression	Umbilical cord blood Pb Mean (SD): 14.4 (8.9) ≥ 10 µg/dL: 53.7% ≥ 25 µg/dL: 9.2%	β (p-value) Birth weight: -0.81 (0.01) Birth length: 0.41 (0.05) Mid-arm circumference: 0.30 (0.05)	Age, sex Note: inclusion of SES did not change the results

Table 4-42 (Continued): Summary of recent epidemiologic studies of associations between Pb exposure indicators and low birth weight and fetal growth.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Data for Pb Biomarkers or other Exposure Indicators	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Llanos and Ronco (2009)	Fetal growth restriction (1,000-2,500g) *note normal birth weights were >3,000g	Term births (37-40 weeks) from non-smoking mothers $N_{growth\ restricted}$=20 $N_{normal\ BW}$=20 Santiago, Chile NS	Cross-sectional study using Mann-Whitney U-test	Placenta Pb Mean (SD) Fetal growth restricted: 0.21 (0.04) µg/g Controls: 0.04 (0.009) µg/g	P-value for Mann-Whitney U-test <0.01	None
Zentner et al. (2006)	Birth weight and length	Singleton births with maternal residence within 5 km of Pb smelter N=55 Santo Amaro, Brazil 2002	Cross-sectional study using linear regression	Umbilical cord blood Pb from delivery Mean (SD): 3.9 (3.6)	Linear regression coefficient (p-value) with umbilical cord blood Pb as the dependent variable in model with only length and weight (units not given for any measurements, assume per 1 µg/dL for cord blood Pb): Length: -0.46 (0.003) Weight: -0.275 (0.048) (i.e., in this study, Pb is assessed as the outcome)	No other variables besides length and weight were included in the model
Janjua et al. (2009)	Low birth weight (≤ 2,500g)	Infants of randomly selected women who planned to deliver between 37-42 weeks N_{LBW}=100 $N_{normal\ BW}$=440 Karachi, Pakistan 2005	Cross-sectional study using binomial regression	Umbilical cord blood Pb Mean (SD): 10.8 (0.2)	Prevalence ratio (95% CI): <10 µg/dL: 1.00 (Ref) ≥ 10 µg/dL: 0.82 (0.57, 1.17)	None

Table 4-42 (Continued): Summary of recent epidemiologic studies of associations between Pb exposure indicators and low birth weight and fetal growth.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Data for Pb Biomarkers or other Exposure Indicators	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Jones et al. (2010)	Low birth weight (<2,500g)	Singleton births ≥ 27 weeks gestation from mothers aged 16-45 yr living in the Shelby County area for at least 5 mo during pregnancy N_{LBW}=10 $N_{normal\ BW}$=92 Tennessee 2006	Cross-sectional study comparing across geometric means (test not specified)	Umbilical cord blood Pb Mean (SD): 2.4 (4.3) Geometric mean: 1.3	Geometric Mean: Low birth weight: 1.2 Normal birth weight: 1.3 p-value for difference: >0.10	None
Örün et al. (2011)	Birth weight and head circumference	Births to mothers not occupationally exposed to toxic metals and living in a suburban but non-industrial area N_{LBW}=9 $N_{normal\ BW}$=135 Turkey NS	Cohort study using Pearson correlation coefficients	Breast milk 2 months post-partum Median: 20.6 µg/L >WHO limit (5 µg/L): 87% Median (IQR) <2,500g: 20.4 (8.5, 27.1) µg/L ≥ 2,500g: 20.6 (11.8, 29.5) µg/L	Correlations for breast milk Pb and z-scores of head circumference Girls: 0.087 Boys: 0.029 Correlations for breast milk Pb and z-scores of birth weight Girls: 0.097 Boys: 0.045 *All p-values for correlations>0.05	None

Table 4-42 (Continued): Summary of recent epidemiologic studies of associations between Pb exposure indicators and low birth weight and fetal growth.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Data for Pb Biomarkers or other Exposure Indicators	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Williams et al. (2007)	Birth weight	Infants from singleton births or the firstborn infant in a set of multiples N=not specified Tennessee 2002	Longitudinal cohort study using hierarchical linear models	Air Pb levels during first trimester of pregnancy Mean (SD): 0.12 µg/m^3 (0.04 µg/m^3)	p-value for multilevel regression of Pb with birth weight: 0.002 Increase of air Pb from 0 to 0.04 relates to a 38g decrease in birth weight Increase of Pb from 0 to 0.13 (maximum) µg/m^3 relates to a 124g decrease in birth weight	Previous preterm birth, previous birth>4000g, pregnancy-induced hypertension, chronic hypertension, oligohydramnios, other maternal risk factors, education, cigarettes/day, black race, Hispanic ethnicity, other race/ethnicity, plurality, infant sex, first trimester SO_2, within 5km of an air monitor, poverty, interaction of poverty and other maternal risk factors, percentage of previous pregnancies that resulted in non-live births

Table 4-42 (Continued): Summary of recent epidemiologic studies of associations between Pb exposure indicators and low birth weight and fetal growth.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Data for Pb Biomarkers or other Exposure Indicators	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Berkowitz et al. (2006)	Low birth weight (<2,500 g and ≥ 37 weeks) Small for gestational age (birth weight ≤ 5th percentile of sex- and gestational week weights for singletons in Idaho)	Singleton infants with 28-45 weeks gestation N_{LBW}=4,297 $N_{normal\ BW}$=162,035 N_{SGA}=7,020 $N_{no\ SGA}$=162,035 Idaho 1970-1981	Cohort study using logistic regression	Three time periods of two locations (unexposed and exposed/near smelter): pre-fire, "high-exposure period" (when a fire happened at the smelter and resulted in damages leading to high air Pb concentrations for 6 mo), and "post-fire" During the time of the fire, estimates of Pb in ambient air were as high as 30 µg/m^3	Term Low birth weight: OR (90% CI) (unexposed location is referent group): Pre-fire: 0.81 (0.55, 1.20) High exposure: 2.39 (1.57, 3.64) Post-fire: 1.28 (0.95, 1.74) Small for gestational age: OR (90% CI) (unexposed location is referent group): Pre-fire: 0.98 (0.73, 1.32) High exposure: 1.92 (1.33, 2.76) Post-fire: 1.32 (1.05, 1.67)	Maternal age, infant sex, first birth, previous miscarriage or abortion

[a]Studies are presented in order of first appearance in the text of this section, which is based on study design and methodological strength.

Multiple studies were conducted that examined the association between maternal blood Pb and birth weight/fetal growth. A study in Vienna, Austria reported an inverse association between maternal blood Pb levels and birth weight but no associations for birth length or head circumference (Gundacker et al., 2010). Similarly, increased maternal blood Pb was associated with decreased birth weight among infants in a study performed in New York (Zhu et al., 2010). No association was observed between maternal blood Pb levels and having an infant who was small for his/her gestational age (SGA). A study in Taiwan examined both maternal and paternal blood Pb levels among those occupationally exposed to Pb and their associations with birth weight and SGA (Chen et al., 2006a). Paternal blood Pb levels were not associated with increased risk of low birth weight or SGA. Higher maternal blood Pb concentration was associated with higher risk of low birth weight and SGA, although not all of the associations were statistically significant. There were small numbers of infants with low birth weight or SGA, especially at the highest blood Pb levels (≥ 20 µg/dL). In California, blood Pb measurements of women during pregnancy were matched with the corresponding birth certificates (Jelliffe-Pawlowski et al., 2006). The adjusted OR for low birth weight that compared women with blood Pb levels ≥ 10 µg/dL to women with levels <10 µg/dL was elevated. However, it was difficult to draw conclusions about the relationship between blood Pb and birth weight due to small numbers (n = 9 for low birth weight) and the consequently wide 95% CI. An association was detected for increased blood Pb and SGA. Women residing in two different towns in Yugoslavia (one with a Pb smelter and one without a Pb smelter) were recruited during their first prenatal visit (Lamb et al., 2008) (study based on previous work by Factor-Litvak et al. (1991)). The mid-pregnancy blood Pb levels were greater in women from the town with a Pb smelter. No association was reported between maternal blood Pb and height or BMI at birth for the infants of these women despite the differences in maternal blood Pb between the two towns. A study of term births in Iran reported no difference in blood Pb levels of women giving birth to a normal weight infant and women giving birth to an infant with low birth weight (Iranpour et al., 2007).

A study examining the association between Pb biomarker levels and birth weight used tibia bone measurements one month post-partum from mothers living in Mexico City (Kordas et al., 2009). Tibia Pb levels were inversely associated with birth weight but not with birth length. This association between Pb and birth weight was not modified by maternal folate consumption or maternal or infant MTHFR genotype, although the association between tibia Pb levels and birth weight was greater in magnitude among women with certain MTHFR SNPs (statistical tests not reported). Another study in Mexico City reported no association between maternal tibia Pb levels and birth weight among girls but reported a positive association for boys (Afeiche et al., 2011). No associations were observed with maternal patella Pb concentration, although among boys,

the relationship was positive but not statistically significant. One of the cohorts used by Afeiche et al. (2011) was also evaluated in another study (Cantonwine et al., 2010b). An inverse association was observed between maternal tibia Pb and birth weight, especially at higher levels (over 15.4 µg/dL). This association was stronger among those mothers with variants of the hemochromatosis Fe gene (HFE).

Multiple studies examined the relationship between Pb level and birth weight using Pb measured from the placenta or umbilical cord. A study performed in Baltimore, MD reported no association between umbilical cord blood Pb concentration and birth weight (Wells et al., 2011a). This study had low blood Pb levels, with only 0.7% of participants having umbilical cord blood Pb measuring >5 µg/dL. In Saudi Arabia, a study was conducted among non-occupationally exposed women (Al-Saleh et al., 2008b). Umbilical cord blood Pb concentrations were low and an association was observed between umbilical cord Pb levels above the 75th percentile (2.475 µg/dL) and head circumference. A study with high Pb concentrations in umbilical cord blood reported an inverse association between Pb levels and birth weight (Atabek et al., 2007). However, no correlation was detected in an analysis restricted to umbilical cord Pb less than 10 µg/dL. No associations with other measures of growth, such as birth length and mid-arm circumference, were detected. Researchers in Chile collected the placentas from term births and compared the Pb levels for those born with normal birth weights to those with low birth weights (Llanos and Ronco, 2009). Pb levels were greater in the placentas of infants with low birth weights. In addition, the authors note that 3 low birth weight infants had extremely high Pb levels in the placentas (>1.5 µg/g) and were excluded from these analyses. A study in Brazil examined Pb levels in umbilical cord blood from term births of women residing within 5 km of a Pb smelter (Zentner et al., 2006). The cord blood Pb level was found to be inversely correlated with length and weight of the infants. Another study recruited women in Pakistan (Janjua et al., 2009). Umbilical cord blood Pb levels were not associated with low birth weight. The study by Iranpour et al. (2007) discussed above investigated the association with umbilical cord blood Pb levels in addition to their examination of maternal whole blood Pb. They again report no difference in cord blood Pb levels between term infants of normal and low birth weight. A study comparing geometric mean umbilical cord blood Pb levels reported no difference in the levels for normal and low birth weight infants born to women living primarily in urban areas of Memphis, TN (Jones et al., 2010). A study previously mentioned that observed an inverse association between maternal tibia Pb and birth weight in Mexico City reported no association between umbilical cord blood Pb concentration and birth weight (Cantonwine et al., 2010b). Finally, a study in Vienna measured Pb in the placenta (Gundacker et al., 2010). A positive correlation was observed between placenta Pb and birth length and weight; however, in the same study, maternal blood Pb was inversely related to birth weight.

A study performed in Turkey examined the relationship between Pb levels in breast milk two months postpartum and size at birth (Örün et al., 2011). No association was observed between breast milk Pb concentration and birth weight or head circumference.

A few studies examined air exposures and reported inverse associations between air Pb concentrations and birth weight. However, a limitation of these studies is the difficulty in assessing if the measured concentrations represent population exposures (see Section 3.5.3). Williams et al. (2007) examined Pb concentrations in the air during the first trimester. The purpose of their study was to demonstrate the use of hierarchical linear models and they used the example of air pollution and birth weight in Tennessee. The model results showed an association between ambient Pb concentration and birth weight, with an estimated decrease in birth weight of 38 grams for every 0.04 $\mu g/m^3$ (i.e., one standard deviation) increase in Pb concentration. Another study of air Pb levels was conducted in Idaho and included two areas over three time periods. One study area was affected by damage to pollution control facilities for a large Pb smelter that led to high Pb emissions during one of the time periods under study (Berkowitz et al., 2006). During the 6 month period when emission controls were affected by the fire damage, estimates of Pb in ambient air were as high as 30 $\mu g/m^3$. Mean birth weight for term births was decreased among infants born to women living in the high exposure area during the period of high exposure compared to those living in the lower exposure area. The difference in birth weight of term births remained, but was reduced, between the two areas during the time period after the exposure ended. During the period of higher exposure, the odds of low birth weight among term births was increased among those living in the higher exposed area compared to those in the lower exposed area, but the odds were not different between the two study areas during the time periods before or after the high level of exposure. An increase in SGA infants (defined as infants with weights less than or equal to the lowest 5th percentile of birth weight for their sex and age) was also associated with living in the higher exposed area during the time period of higher exposure. The odds of SGA infants decreased during the time period after the exposure but the odds were still elevated compared to those residing in the lower exposed area.

Evidence from previous toxicological studies has shown an association between gestational Pb exposure and reduced birth weight (U.S. EPA, 2006b). More recent studies have reported conflicting results. Wang et al. (2009e) demonstrated a statistically significant decrease in fetal body weight and body length of Wistar rats after maternal exposure to 250 ppm Pb acetate during gestation days 1-10, 11-20, or 1-20. The greatest decrease in fetal body weight and length was observed in the group exposed to Pb during gestation days 1-20 followed by the group exposed to Pb during gestation days 11-20. Teijón et al. (2006) observed that when pregnant dams were administered 200 ppm or

400 ppm Pb acetate in drinking water, litter weight was significantly decreased (400 ppm Pb only) versus controls due to significant decrements in female pup birth weight; male birth weight was unaffected. The results of these studies indicate that as Pb exposure increases, the body weight of exposed offspring decreases. Massó-González and Antonia-García (2009) also observed an 8-20% decrease in body weight of pups from rat dams given 300 mg/L Pb acetate in drinking water (exposure during gestation and lactation resulting in mean blood Pb level of 22.8 µg/dL), but no changes in body length were reported.

In summary, associations were observed between Pb and low birth weight in epidemiologic studies of maternal bone Pb and studies of Pb air exposures and birth weight. The associations were less consistent when using maternal blood Pb or umbilical cord and placenta Pb as the exposure measurement although some studies did demonstrate associations. Epidemiologic studies of Pb and fetal growth face multiple limitations. One limitation is the cross-sectional nature of many studies. These do not allow an understanding of the temporality for Pb and fetal growth. In addition, some studies suffer from small sample size. The studies of air Pb levels and birth weight, one of which involved air Pb concentrations as high as 30 µg/m^3, demonstrate positive associations but are limited in that exposure levels are unknown and certain size fractions have less certain relationships with population exposure levels (see Chapter 3). Also, many of the studies controlled for important confounders, such as parity and gestational age, but adjustment in some studies was lacking. Toxicological studies observed an association between gestational Pb exposure and reduced birth weight with moderate (22.8 µg/dL blood Pb level) to higher dose Pb (400 ppm Pb acetate in drinking water).

4.8.3 Effects on Male Reproductive Function

The 2006 Pb AQCD (U.S. EPA, 2006b) reported on male Pb exposure or biomarker levels and reproductive functions in males as measured by sperm count/motility/morphology, time to pregnancy, reproductive history, and chromosomal aberrations. Despite limitations, most of the studies found slight associations between high blood Pb levels (i.e., ≥ 45 µg/dL) and reduced male fecundity or fertility (U.S. EPA, 2006b). Evidence reviewed in the 1986 Pb AQCD (U.S. EPA, 1986a) also demonstrated that Pb exposure affects male reproductive function in humans and experimental animals. Recently published research has continued to support an association between Pb and sperm/semen production, quality, and function. Studies of Pb and male reproductive function are described in the sections below. The studies presented in the following text and tables are grouped by study design and methodological strength.

4.8.3.1 Effects on Sperm/Semen Production, Quality, and Function

Multiple epidemiologic and toxicological studies have examined the relationship between Pb and sperm and semen production, quality, and function. These studies are summarized in the text below. In addition, recent epidemiologic studies are included in Table 4-43. All epidemiologic studies were cross-sectional with concurrent measurements of Pb levels in biological samples and sperm-related outcomes.

Table 4-43 Summary of recent epidemiologic studies of associations between Pb biomarker levels and effects on sperm and semen.

Reference[a]	Study Population Study Location Years	Methodological Details	Pb Biomarker Data Mean (SD) in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Hsu et al. (2009b)	Men working at a battery plant N=80 Taiwan NS	Occupational cohort study (cross-sectional) using ANOVA and linear regression	Blood Pb: 42 Categorized into 3 groups: <25 µg/dL, 25-45 µg/dL, >45 µg/dL	p-values for difference across the three groups were <0.05 for: sperm head abnormalities, sperm neck abnormalities, sperm chromatin structure assay (αT, COMPαT) p-values for difference across the three groups were >0.05 for: semen volume, sperm count, motility, sperm tail abnormalities, sperm immaturity, computer-assisted semen analysis, % sperm with ROS production Coefficients for regression analysis with blood Pb: Morphologic abnormality: 0.271 (p-value <0.0001) **Head abnormality:** 0.237 (p-value 0.0002) **αT:** 1.468 (p-value 0.011) **COMPαT**: 0.233 (p-value 0.21)	Smoking status

Table 4-43 (Continued): Summary of recent epidemiologic studies of associations between Pb biomarker levels and effects on sperm and semen.

Reference[a]	Study Population Study Location Years	Methodological Details	Pb Biomarker Data Mean (SD) in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Kasperczyk et al. (2008)	Healthy, non-smoking, fertile men who worked at the Zn and Pb Metalworks $N_{controls}$=14 $N_{low\ exposure}$=20 $N_{high\ exposure}$=29 Poland NS	Occupational cohort study (cross-sectional) using Kruskal-Wallis ANOVA and Spearman's coefficient for non-parametric correlation	Blood Pb Categorized as High exposure workers : 53.1 (2.05) Range: 40-81 µg/dL Low exposed workers: 34.7 (0.83) Range: 25-40 µg/dL Controls (office workers with no history of occupational Pb exposure): 8.47 (0.54) Seminal plasma Pb High exposure workers: 2.02 (0.23) Low exposure workers: 2.06 (0.40) Controls: 1.73 (0.16)	Mean (SE) **Sperm volume (mL)** Controls: 2.94 (0.32) Low exposure: 2.89 (0.22) High exposure: 2.98 (0.22) (p-value for ANOVA: 0.993) **Sperm cell count (mln/mL)** Controls: 43.1 (7.0) Low exposure: 44.6 (10.1) High exposure: 42.2 (5.86) (p-value for ANOVA: 0.400) **Normal morphology (%)** Controls: 63.3 (2.7) Low exposure: 57.3 (2.5) High exposure: 58.4 (2.1) (p-value for ANOVA: 0.266) **Progressively motile sperm after 1 h (%)** Controls: 16.4 (3.2) Low exposure: 14.8 (2.6) High exposure: 10.5 (1.9) (p-value for ANOVA: 0.217) **Motile sperm after 24 h (%)** Controls: 4.4 (1.8) Low exposure: 7.3 (1.7) High exposure: 3.1 (0.8) (p-value for ANOVA: 0.188) p-value for correlation between blood Pb and sperm cell motility after 1 h: 0.011	None

Table 4-43 (Continued): Summary of recent epidemiologic studies of associations between Pb biomarker levels and effects on sperm and semen.

Reference[a]	Study Population Study Location Years	Methodological Details	Pb Biomarker Data Mean (SD) in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Naha and Manna (2007)	Non-occupationally exposed controls and occupationally exposed workers $N_{controls}$=50 $N_{low\ exposure}$=30 $N_{high\ exposure}$=20 Bangalore, India NS	Occupational cohort study using ANOVA, Student's t-test, and Scheffe's F-test	Categorized by work history as controls, low exposure (7-10 yr of exposure for 8 h/day) and high exposure (>10 yr of exposure for 8 h/day) Blood Pb measurement Controls: 10.25 (2.26) Low exposure : 50.29 (3.45) High exposure: 68.26 (2.49) Semen Pb measurement Controls: 2.99 (0.76) Low exposure: 15.85 (1.95) High exposure: 25.30 (2.28)	p-values for difference across the three groups for mean values of semen profiles were <0.01 for: liquefaction time, seminal volume, sperm count, sperm DNA hyploidy, sperm morphological abnormality, sperm motility, sperm ATPase activity, seminal plasma fructose, seminal plasma total protein, seminal plasma free amino acid, seminal plasma cholesterol	None
Naha and Chowdhury (2006)	Men aged 31-45 that were non-occupationally exposed controls and occupationally exposed workers) $N_{controls}$=50 $N_{low\ exposure}$=30 $N_{high\ exposure}$=50 Kolkata, India NS	Occupational cohort study using ANOVA, Student's t-test, and Scheffe's F-test	Categorized by work history as controls, low exposure (7-10 yr of exposure for 8 h/day) and high exposure (>10 yr of exposure for 8 h/day) Blood Pb measurement Controls 13.62 (2.45) Low exposure 48.29 (4.91) High exposure 77.22 (1.25) Semen Pb measurement Controls 3.99 (1.36) Low exposure 10.85 (0.75) High exposure 18.30 (2.08)	p-values for difference across the three groups for mean values of semen profiles were <0.01 for: sperm count, sperm protein, sperm DNA hyploidy, sperm DNA, sperm RNA, sperm viability, sperm membrane lipid peroxidation, seminal plasma total ascorbate, seminal plasma DHAA, sperm ATPase activity, sperm motility, sperm velocity, seminal plasma fructose	None

Table 4-43 (Continued): Summary of recent epidemiologic studies of associations between Pb biomarker levels and effects on sperm and semen.

Reference[a]	Study Population Study Location Years	Methodological Details	Pb Biomarker Data Mean (SD) in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Telisman et al. (2007)	Men aged 19-55 yr, never occupationally exposed to metals and going to a clinic for infertility examination or for semen donation to be used for artificial insemination N=240 Croatia 2002-2005	Cross-sectional study using linear multiple regression	Blood Pb Median: 4.92 (range 1.13-14.91)	Standardized regression coefficients for log blood Pb (units not given) **Immature sperm:** 0.13 (p-value <0.07) **Pathologic sperm:** 0.31 (p-value <0.0002) **Wide sperm:** 0.32 (p-value <0.0001) **Round sperm:** 0.16 (p-value <0.03) Coefficients and p-values not given if not statistically significant: semen volume, sperm concentration, slow sperm, short sperm, thin sperm, amorph sperm	Cd, Cu, Zn, Se, age, smoking status, alcohol use

Table 4-43 (Continued): Summary of recent epidemiologic studies of associations between Pb biomarker levels and effects on sperm and semen.

Reference[a]	Study Population Study Location Years	Methodological Details	Pb Biomarker Data Mean (SD) in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Meeker et al. (2008)	Men aged 18-55 yr going to infertility clinics (distinction not made between clinic visits for male or female fertility issues) N=219 Michigan NS	Cross-sectional study using multiple logistic regression	Blood Pb Median: 1.50 (IQR 1.10, 2.00)	OR (95% CI) for having below reference-level semen parameters Concentration 1st quartile: 1.00 (ref) 2nd quartile: 0.88 (0.32, 2.44) 3rd quartile: 2.58 (0.86, 7.73) 4th quartile: 1.16 (0.37, 3.60) Motility 1st quartile: 1.00 (ref) 2nd quartile: 1.04 (0.43, 2.53) 3rd quartile: 1.95 (0.70, 5.46) 4th quartile: 1.66 (0.64, 4.29) Morphology 1st quartile: 1.00 (ref) 2nd quartile: 0.83 (0.37, 1.87) 3rd quartile: 1.41 (0.54, 3.67) 4th quartile: 1.18 (0.50, 2.79) Models with adjustment for multiple metals Concentration 1st quartile: 1.00 (ref) 2nd quartile: 0.89 (1.57, 2.89) 3rd quartile: 3.94 (1.15, 13.6) 4th quartile: 2.48 (0.59, 10.4)	Age, smoking status Models with multiple metals included: smoking status, Mo, Mn, Cd, and Hg Considered but did not include: BMI, race

Table 4-43 (Continued): Summary of recent epidemiologic studies of associations between Pb biomarker levels and effects on sperm and semen.

Reference[a]	Study Population Study Location Years	Methodological Details	Pb Biomarker Data Mean (SD) in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Slivkova et al. (2009)	Men aged 22-48 yr undergoing semen analysis at an infertility clinic N=47 NS	Cross-sectional study using correlation	Semen Pb 1.49 (0.40) mg/kg	Correlation between Pb and flagellum ball : -0.39 (p-value not given) *correlations not given for any other sperm pathological changes (therefore assume not statistically significant): broken flagellum, separated flagellum, separated flagellum, small heads, retention of cytoplasmic drop, other pathological spermatozoa, large heads, acrosomal changes, and knob twisted flagellum	None
Mendiola et al. (2011)	Men attending infertility clinics and classified as either normal sperm (controls) or oligo-astheno-teratozoospermia (cases) based on WHO semen quality criteria $N_{controls}$=31 N_{cases}=30 Spain 2005-2007	Case-control study using multiple linear regression	Seminal plasma: 2.90 (IQR 2.70, 3.20) Whole blood: 9.50 (IQR 7.50, 11.90) Blood plasma: 2.90 (IQR 2.70, 3.10) Cases: Seminal plasma: 3.0 (0.3) Whole blood: 9.8 (2.3) Blood plasma: 2.9 (0.2) Controls: Seminal plasma: 2.9 (0.3) Whole blood: 9.7 (2.3) Blood plasma: 2.9 (0.3)	β (95% CI) Sperm concentration Seminal plasma: -1.0 (-3.1, 2.3) Whole blood: -0.2 (-1.7, 1.6) Blood plasma: 0.08 (-4.1, 5.2) % Immotile sperm Seminal plasma: 1.5 (0.37, 1.9) Whole blood: 0.05 (-0.32, 0.43) Blood plasma: -0.49 (-1.8, 0.62) % Morphologically normal sperm Seminal plasma: -0.54 (-3.1, 2.0) Whole blood: -0.31 (-1.5, 0.89) Blood plasma: -0.08 (-3.5, 3.4) *Units not given (assume 1 µg/dL) Note: No correlation in Pb levels among bloods or seminal plasma. There was correlation between Pb and other metals (Cd and Hg) within each body fluid. Other metals were not controlled for in models	Age, BMI, number of cigarettes/day

[a]Studies are presented in order of first appearance in the text of this section, which is based on study design and methodological strength.

International epidemiologic studies of men occupationally exposed to Pb have reported on associations between Pb exposure or biomarker levels and sperm count and quality and semen quality. In most of these occupational studies, mean blood Pb levels over 40 µg/dL have been reported for individuals occupationally exposed to Pb. In addition, they did not control for other potential occupational exposures. A study performed in Taiwan among men with high levels of blood Pb reported that men with blood Pb levels of 25-45 µg/dL and higher had increased sperm head abnormalities, increased sperm DNA denaturation, and increased sensitivity to denaturation compared to men with lower blood Pb levels (Hsu et al., 2009b). No difference was detected between three Pb exposure groups in semen volume, sperm count, motility, velocity, and reactive oxygen species (ROS) production. A similar study in Poland included employees exposed to Pb and compared them with a group of male office workers (Kasperczyk et al., 2008). Pb levels measured in seminal fluid were slightly higher among those in the exposed groups although they were not statistically different from the levels in the control group. No difference was observed for semen volume, sperm count, or sperm morphology among the groups. Sperm motility was lower in the highest exposure group (40-81 µg/dL) compared to both the control (no history of exposure) and moderate exposure (25-40 µg/dL) groups. Lipid peroxidation, which can induce tissue damage in sperm via ROS, was greater in the highest exposure group compared to the controls. Studies performed in India (Naha and Manna, 2007; Naha and Chowdhury, 2006) reported that men in the highest exposure group (men working in battery or paint manufacturing plants for 10-15 years for 8 hours/day) had mean blood Pb levels of 77.22 µg/dL (Naha and Chowdhury, 2006) and 68.26 µg/dL (Naha and Manna, 2007). Control groups in these studies (those without occupational Pb exposure) had mean blood Pb levels below 15 µg/dL. Increases in levels of Pb in semen were also noted across exposure groups. Both studies report decreases in sperm count and in sperm velocity and motility with increasing Pb exposure. Higher Pb exposure was also associated with greater hyploidy of sperm DNA and morphologic abnormalities (Naha and Manna, 2007; Naha and Chowdhury, 2006). Decreased viability and increased lipid peroxidation were detected (Naha and Chowdhury, 2006).

A few studies examined blood or seminal plasma Pb levels and semen quality of men at infertility clinics (Mendiola et al., 2011; Slivkova et al., 2009; Meeker et al., 2008; Telisman et al., 2007). In general, these men had lower levels of Pb biomarkers than men who were occupationally exposed, but the studies are limited by the strong possibility of selection bias related to the recruitment of men attending infertility clinics. A study performed in Croatia recruited men attending a clinic for infertility examination or to donate semen for use in artificial inseminations, who had never been occupationally exposed to metals and therefore had lower blood Pb levels than the occupational studies (although leaded gasoline was still sold during the study period) (Telisman et al., 2007).

Increased blood Pb was associated with increased percentages of pathologic sperm, wide sperm, and round sperm. There was also a slight increase in immature sperm although it was not statistically significant. Similar results were seen when other biomarkers for Pb (erythrocyte protoporphyrin and δ-aminolevulinic acid dehydratase [ALAD]) were used instead. This study controlled for multiple potential confounders, including other metals. Meeker et al. (2008) detected no associations between higher blood Pb and semen concentration, morphology, or motility (although a slight positive trend was observed between higher Pb levels and motility in unadjusted models). In models that included multiple metals, blood Pb was associated with being below the WHO limit of sperm concentration levels (less than 20 million sperm/mL), although the 95% CI was wide for the 4th quartile of Pb levels and included the null. The precision of estimates in this study was extremely low. Slivkova et al. (2009) reported a negative correlation between semen Pb and pathological changes in sperm (specifically, flagellum ball), but no correlations were observed for other alterations in the sperm. Another study reported a positive association between seminal plasma Pb concentration and percentage of immotile sperm, but this analysis did not adjust for exposure to other metals reported to be correlated with Pb concentration in the seminal plasma (Mendiola et al., 2011). No association was observed for seminal plasma Pb concentration and sperm concentration or percentage of morphologically normal sperm. Additionally, neither Pb levels in whole blood nor plasma were associated with sperm concentration, percentage of immotile sperm, or percentage of morphologically normal sperm.

Extensive evidence in the toxicological literature demonstrates that Pb exposure is detrimental to the quality and overall health of testicular germ cells, affecting sperm and semen quality and production. Earlier Pb AQCDs contained studies of Pb-induced decreased sperm counts, decreased sperm production rate, and dose-dependent suppression of spermatogenesis in adult rodents with 30 day drinking water Pb exposure [(Sokol and Berman, 1991), blood Pb level 35 and 37 µg/dL; (Sokol et al., 1985), blood Pb level 34 µg/dL; (Sokol, 1989), blood Pb levels <43 µg/dL]. Chronic Pb treatment (15 weeks) of adult male rabbits, resulting in a blood Pb of 24 µg/dL, induced statistically significant decrements in semen quality and greater testicular pathology (Moorman et al., 1998) with dosing by subcutaneous injection, loading dose of 0.2-3.85 mg/kg BW Monday (M), Wednesday (W) and Fridays (F) weeks 6-10, followed by maintenance dose of 0.13-2.0 mg/kg BW Pb acetate MWF over weeks 11-20 of the study. The 2006 Pb AQCD also cited studies in which sperm from Pb exposed rats yielded lower rates of fertilization when used for in vitro fertilization of eggs harvested from unexposed females [(Sokol et al., 1994), blood Pb level 33-46 µg/dL].

Recent studies corroborate earlier findings that Pb alters sperm parameters such as sperm count, viability, motility, and morphology. Anjum et al. (2010) exposed 50 day old male

albino Wistar/NIN rats to Pb acetate (273 or 819 mg/L in drinking water, 500 or 1,500 ppm, respectively) for 45 days. Affected endpoints included reduced epididymal sperm count, motile sperm, and viable sperm, indicating decreased sperm production and quality. Anjum et al. (2010) did not report blood Pb values. Wistar/NIN rats (1,500 ppm Pb acetate in drinking water for 70 days) supplemented with the herb Centella asiatica (Sainath et al., 2011) had significant attenuation of the Pb-induced changes observed by Anjum et al. (2010). Pillai et al. (2012) found gestational and lactational treatment with Pb acetate in Charles Foster rats (subcutaneous injection of 0.05 mg/kg BW/day) induced effects on sperm in adults (PND65) including significant decreases in testicular sperm count, epididymal sperm count, and sperm motility. These findings are consistent with Pb-associated effects on sperm and male reproductive organs in wildlife from the ecological literature including deer, Asian earthworms, rainbow trout, marine worms, and the fathead minnow (see Sections 6.3.12.1 and 6.4.12.1 from the ecology terrestrial and aquatic reproduction sperm sections).

Pb exposure has been shown to affect the male reproductive organs, as is seen with histological or morphological changes. Studies included in previous Pb AQCDs showed that histological and ultrastructural damage to the testes or seminiferous tubules was seen in non-human primates with chronic oral Pb exposure (daily Pb exposure, gelatin capsule; control plus 3 treatment groups) during three different time periods: (1) infancy (PND0-PND400), resulting in a maximum blood Pb level of 36 μg/dL, (2) post-infancy (PND300 up to 10 years of age), resulting in a maximum blood Pb level of 33 μg/dL, and (3) lifetime (PND0 up to 10 years of age), resulting in a maximum blood Pb level of 32 μg/dL (Foster et al., 1998; Singh et al., 1993a). Rodent studies using i.p. injections of Pb also showed ultrastructural damage to structures involved in spermatogenesis (blood Pb level after i.p. injection treatment for 16 days: 7.4 μg/dL) (Murthy et al., 1995). More recently, Salawu et al. (2009) observed a decrease in absolute testicular weight after Pb exposure (adult male SD rats, 10,000 ppm Pb acetate in drinking water for 8 weeks). Anjum et al. (2010) reported decreased testicular and epididymal weights of male rats exposed to Pb acetate (500 or 1,500 ppm Pb acetate in drinking water for 45 days) which were significantly attenuated with Pb co-exposure to the herb *Centella asiatica* (Sainath et al., 2011). Pb induced morphological abnormalities in sperm in a concentration-dependent manner (Allouche et al., 2009; Oliveira et al., 2009; Salawu et al., 2009; Shan et al., 2009; Tapisso et al., 2009; Massanyi et al., 2007; Wang et al., 2006a). Sperm abnormalities reported after Pb exposures were amorphous sperm head, abnormal tail, and abnormal neck. Dong et al. (2009) reported decreased epididymis and body weights in mice after an 8-week exposure to 6,000 ppm Pb acetate in drinking water (adult male Kunming mice, 8-week exposure). Oliveira et al. (2009) observed a negative correlation between Pb dose and intact acrosomes (8 week old ICR-CD1 mice, subcutaneous injection of 74 and 100 mg $PbCl_2$/kg body weight for four consecutive days). Rubio et al.

(2006) (adult mice treated with i.p. injections of 8, 16 or 24 mg/kg of Pb acetate for 35 days) and Biswas and Ghosh (2006) (adult male Wistar rats, i.p. injection of 8 mg/kg body weight Pb acetate for 21 days) also observed a Pb-induced decrease in seminal vesicle and ventral prostate weights. Rubio et al. (2006) reported that Pb acetate, in a concentration-dependent manner (8-24 mg/kg body weight), reduced the length of certain stages of the spermatogenic cycle of rat seminiferous tubules and thus affected spermatogenesis. Oral Pb acetate exposure (25 mg/kg bw in drinking water for 3 months, resulting in blood Pb level of 5.3 µg/dL) to adult male albino rats produced significant histological seminiferous tubule damage (epithelium, spermatocytes, acrosomes) that was attenuated with ascorbic acid treatment (Pb exposure + 100 mg/kg bw/day ascorbic acid, resulting in blood Pb level of 4.7 µg/dL) (El Shafai et al., 2011). However, the majority of studies did not observe a statistically significant difference in body weight or reproductive organ weights after Pb exposure at the doses used in the studies. Not all of the aforementioned studies observed changes in every parameter. This may be due to the use of different strains or species, chemical form of the Pb compound administered, dosage schedule, duration of exposure, and age of animals at the time of the study (Oliveira et al., 2009).Data from recent studies suggested that the generation of ROS in the male reproductive tissues, which can then affect antioxidant defense systems of cells (Pandya et al., 2010) (adult male Charles Foster rats, Pb acetate 0.025 mg/kg body weight/day i.p. for 8 weeks) contributes to the mode of action of Pb damage to the male reproductive organs and sperm or semen. Salawu et al. (2009) observed a statistically significant increase in malondialdehyde (MDA, oxidative stress marker) and a significant decrease in the activity of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) in plasma and testes of adult male Sprague Dawley rats after administration of 10,000 ppm Pb acetate in drinking water for 8 weeks. Supplementation with tomato paste (used as a source of antioxidants) reduced Pb-induced ROS production and prevented the Pb-induced increase in MDA formation and decrease in SOD and CAT activity. Furthermore, co-treatment of Pb with substances that are known to have antioxidant properties [i.e., tomato paste, Maca (*Lepidium meyenii*), and ascorbic acid] prevented the Pb-induced reduction in sperm count, sperm motility, and sperm viability (Salawu et al., 2009; Shan et al., 2009; Madhavi et al., 2007; Rubio et al., 2006; Wang et al., 2006a).

Recent studies continue to demonstrate that Pb may be directly toxic to mature spermatozoa (adult Algerian mice, Pb acetate 21.5 mg/kg BW/every other day i.p. for 11 or 21 days and adult NMRI mice, 6,000 ppm Pb chloride in drinking water for 16 weeks, respectively) (Tapisso et al., 2009; Hernandez-Ochoa et al., 2006) as well as primary spermatocytes (adult male Wistar rats treated with drinking water containing 250mg/L Pb acetate and adult albino rats, 10 mg/kg BW Pb chloride i.p. once daily for 8 weeks) (Nava-Hernandez et al., 2009; Rafique et al., 2009). Nava-Hernandez et al. (2009) exposed two groups of adult male rodents to Pb via drinking water (L1 and L2, 250mg/L

or 500 mg/L Pb acetate starting at PND60 for 90 days). They found significant increases in spermatid DNA damage with Pb exposure. In their study, all Pb-treated animals had blood Pb levels statistically significantly higher than controls (L1:19.54 µg/dL and L2:21.90 µg/dL); no statistically significant difference in blood Pb levels existed between the two Pb exposure groups likely because the L2 group drank less water than did the L1 group. Piao et al. (2007) reported that Pb exposure caused DNA damage to sperm; the Pb exposed group had a blood Pb of 6.7 µg/dL. Piao et al. (2007) also examined the effect of Zn supplementation on Pb-induced sperm aberrations and found that the proportion of abnormal sperm was statistically significantly higher in the Pb group and the Pb+Zn group (25 mg/kg Pb acetate i.p., 4 mg/kg Zn acetate i.p., both Pb acetate and Zn acetate, once every two days, for 2 weeks) than in controls. However, the proportion of abnormal sperm in Pb+Zn group was statistically significantly lower than in Pb alone group. Hernandez-Ochoa et al. (2006) reported that Pb reaches the sperm nucleus in the epididymis of mice chronically exposed (16 weeks in adult animals) to Pb (resulting in mean blood Pb of 75.6 µg/dL) by binding to nuclear sulfhydryl groups from the DNA-protamine complex, increasing sperm chromatin condensation, and thereby interfering with the sperm maturation process without altering sperm quality parameters. Tapisso et al. (2009) observed a statistically significant increase in the number of micronuclei and frequency of sister chromatid exchange with increasing treatment duration in adult male Algerian mice administered 21.5 mg/kg body weight Pb acetate by i.p. injection (every other day for 11 or 21 days). Nava-Hernandez (2009) reported a concentration-dependent increase in DNA damage in rat primary spermatocytes after a 13-week exposure period to Pb acetate in drinking water (resulting in mean blood Pb levels between 19.5 and 21.9 µg/dL). Rafique et al. (2009) reported degenerative changes from pyknosis to apoptosis in primary spermatocytes (adult albino rats, 10 mg/kg BW Pb chloride i.p. once daily for 8 weeks). Hepatic expression of spermatogenic genes was transiently down-regulated in 8 week old male Wistar-Kyoto (WKY) rats in response to Pb nitrate (100 µmol single i.v. injection) 3 hours after injection and recovered to baseline by 12 hours (Nemoto et al., 2011); this effect was not seen in the stroke-prone spontaneously hypertensive rats, which are from a WKY background, or in Sprague-Dawley rats, demonstrating strain specificity.

Pb-induced apoptosis in germ cells within the seminiferous tubules is another suggested mechanism by which Pb exerts its toxic effects on sperm production and function (Wang et al., 2006a) (Kunming mice, 2,000 ppm Pb acetate in drinking water for 14-42 days). Dong et al. (2009) reported a exposure concentration-related increase in apoptosis in spermatogonia and spermatocytes of Kunming mice after exposure to 1,500–6,000 ppm Pb acetate in drinking water. Pb-induced testicular germ cell apoptosis was associated with up-regulation of genes involved in the signal pathway of MAPK and death receptor signaling pathway of Fas. For instance, up-regulation of K-ras and Fas expressions was

concomitant with activation of c-fos and active caspase-3 proteins. Wang et al. (2006a) observed a Pb exposure concentration-dependent increase in the expression of apoptotic markers TGFβ1 and caspase-3 in spermatogenic cells, Sertoli cells, and Leydig cells. Shan et al. (2009) (20 mg/kg BW intragastric Pb acetate for 6 weeks) also reported a statistically significant increase in mRNA expression and protein levels of Fas, Fas-L and caspase-3 after Pb treatment. Supplementation with ascorbic acid inhibited or reduced the Pb-induced apoptosis in germ cells and protected testicular structure and function (El Shafai et al., 2011; Shan et al., 2009; Wang et al., 2006a) suggesting ROS generation is a major contributing factor in decreased male fertility observed after chronic Pb exposure.

Similar to the results summarized in previous Pb AQCDs, recent epidemiologic and toxicological studies indicate that Pb exposure has effects on sperm, semen, and male reproductive organs. Consistent toxicological evidence from multiple labs with multiple species of animals showed decrements in sperm or semen quality with Pb exposure including decreased sperm counts, decreased sperm production rate, and a dose-dependent suppression of spermatogenesis. Histological damage to rodent sperm and ultrastructural damage to rodent and non-human primate seminiferous tubules has been reported with Pb exposure. Sperm from Pb-exposed male rodents used for in vitro fertilization of eggs from unexposed females yielded a lower rate of fertilization. Also, direct effects of Pb on rodent sperm DNA have been reported in rodents with drinking water exposure. The toxicological findings cross species and are seen in wildlife including deer, earthworms, rainbow trout, marine worms, and fathead minnow.
In studies of men exposed to Pb in occupational settings, associations were observed between blood Pb levels of at least 25 µg/dL and sperm count and quality. Multiple epidemiologic studies of occupational cohorts included control populations with high blood Pb levels (close to or greater than 10 µg/dL), which makes identification of effects at lower levels difficult. Occupational studies had limited consideration for potential confounding factors, such as other workplace exposures. An epidemiologic study of men attending a clinic for purposes of infertility exam or semen donation demonstrated an inverse relationship between Pb levels and sperm and semen quality (Telisman et al., 2007). This study also controlled for other metals in the analyses. Other studies of men at infertility clinics had greater imprecision in their estimates, less control for confounding (such as other metals), and/or small sample sizes. Additionally, studies limited to men at infertility clinics may suffer from selection bias and are not generalizable.

4.8.3.2 Effects on Hormone Levels

The 2006 Pb AQCD (U.S. EPA, 2006b) provided evidence that Pb acts as an endocrine disruptor in males at various points along the hypothalamic-pituitary-gonadal axis. The

2006 Pb AQCD also reported inconsistencies in the effects of Pb exposure on circulating testosterone levels. Recent epidemiologic and toxicological studies are reported below. Epidemiologic studies are summarized in Table 4-44. Epidemiologic studies were cross-sectional; biological samples used for the measurement of Pb were measured concurrently with hormone levels. One study estimated cumulative blood Pb.

Table 4-44 Summary of recent epidemiologic studies of associations between Pb exposure indicators and hormones for males.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Data for Pb Biomarker or other Exposure Indicator	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Telisman et al. (2007)	FSH, LH, testosterone, estradiol, prolactin	Men aged 19-55 yr, never occupationally exposed to metals and going to a clinic for infertility examination or for semen donation to be used for artificial insemination N=240 Croatia 2002-2005	Cross-sectional study using linear multiple regression	Blood Pb (µg/dL) Median: 4.92 (range 1.13-14.91)	Standardized regression coefficients for log blood Pb (units not given) **Testosterone**: 0.21 (p-value <0.003) **Estradiol**: 0.22 (p-value <0.0008) **Prolactin**: - 0.18 (p-value <0.007) Note: Coefficients and p-values not given if not statistically significant (LH, FSH)	Age, smoking status, alcohol use, Cd, Cu, Zn, Se An interaction between Pb and Cd was included in models for testosterone

Table 4-44 (Continued): Summary of recent epidemiologic studies of associations between Pb exposure indicators and hormones for males.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Data for Pb Biomarker or other Exposure Indicator	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Meeker et al. (2010)	FSH, LH, inhibin B, testosterone, SHBG, FAI, testosterone/LH	Men aged 18-55 yr going to infertility clinics (distinction not made between clinic visits for male or female fertility issues) N=219 Michigan NS	Cross-sectional study using multiple linear regression	Blood Pb (µg/dL) Median: 1.50 (IQR 1.10, 2.00)	Regression coefficients (95% CI) **FSH** 1st quartile: 0 (ref) 2nd quartile: 0.13 (-0.10, 0.37) 3rd quartile: 0.10 (-0.15, 0.35) 4th quartile: 0.07 (-0.18, 0.31) **LH** 1st quartile: 0 (ref) 2nd quartile: 0.004 (-0.20, 0.21) 3rd quartile: 0.13 (-0.09, 0.35) 4th quartile: 0.88 (-0.14, 0.29) **Inhibin B** 1st quartile: 0 (ref) 2nd quartile: -6.45 (-27.2, 14.3) 3rd quartile: -4.62 (-26.6, 17.4) 4th quartile: -7.79 (-29.0, 13.4)	Age, BMI, current smoking Considered but did not include: race, income, season

Table 4-44 (Continued): Summary of recent epidemiologic studies of associations between Pb exposure indicators and hormones for males.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Data for Pb Biomarker or other Exposure Indicator	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Meeker et al. (2010) (Continued)	FSH, LH, inhibin B, testosterone, SHBG, FAI, testosterone/LH	Men aged 18-55 yr going to infertility clinics (distinction not made between clinic visits for male or female fertility issues) N=219 Michigan NS	Cross-sectional study using multiple linear regression	Blood Pb (µg/dL) Median: 1.50 (IQR 1.10, 2.00)	**Testosterone** 1st quartile: 0 (ref) 2nd quartile: 28.6 (-6.82, 64.1) 3rd quartile: 15.8 (-21.8, 53.3) 4th quartile: 39.9 (3.32, 76.4) **SHBG** 1st quartile: 0 (ref) 2nd quartile: -0.01 (-0.16, 0.15) 3rd quartile: 0.04 (-0.12, 0.21) 4th quartile: 0.07 (-0.10, 0.23) **FAI** 1st quartile: 0 (ref) 2nd quartile: 0.8 (-0.04, 0.20) 3rd quartile: 0.03 (-0.10, 0.17) 4th quartile: 0.08 (-0.05, 0.21) **Testosterone /LH** 1st quartile: 1.00 (ref) 2nd quartile: 0.07 (-0.16, 0.30) 3rd quartile: -0.05 (-0.29, 0.19) 4th quartile: 0.07 (-0.17, 0.31)	Age, BMI, current smoking Considered but did not include: race, income, season

Table 4-44 (Continued): Summary of recent epidemiologic studies of associations between Pb exposure indicators and hormones for males.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Data for Pb Biomarker or other Exposure Indicator	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Mendiola et al. (2011)	FSH, LH, testosterone	Men attending infertility clinics and classified as either normal sperm (controls) or oligo-astheno-teratozoospermia (cases) based on WHO semen quality criteria N_{cases}=30 $N_{controls}$=31 Spain 2005-2007	Case-control study using multiple linear regression	Mean (IQR) (µg/dL) Seminal plasma: 2.90 (2.70, 3.20) Whole blood: 9.50 (7.50, 11.90) Blood plasma: 2.90 (2.70, 3.10) Mean (SD) Cases: Seminal plasma: 3.0 (0.3) Whole blood: 9.8 (2.3) Blood plasma: 2.9 (0.2) Controls: Seminal plasma: 2.9 (0.3) Whole blood: 9.7 (2.3) Blood plasma: 2.9 (0.3)	Linear regression β (95% CI) FSH Seminal plasma: 0.05 (-0.24, 0.39) Whole blood: 0.04 (-0.03, 0.04) Blood plasma: -0.20 (-0.64, 0.25) LH Seminal plasma: 0.14 (-0.13, 0.41) Whole blood: 0.05 (-0.05, 0.07) Blood plasma: -0.07 (-0.49, 0.31) Testosterone Seminal plasma: 0.11 (-0.10, 0.31) Whole blood: 0.01 (-0.05, 0.02) Blood plasma: -0.12 (-0.40, 0.14) *Units not given (assume 1 µg/dL)	Age, BMI, number of cigarettes/day

Table 4-44 (Continued): Summary of recent epidemiologic studies of associations between Pb exposure indicators and hormones for males.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Data for Pb Biomarker or other Exposure Indicator	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Hsieh et al. (2009a)	FSH, LH, testosterone, inhibin B	Workers at a Pb-acid battery factory with annual blood Pb measures N=181 Taiwan 1991-NS	Longitudinal occupational cohort study using multivariate linear regression	Concurrent blood Pb: <10 µg/dL: 11.6% >40 µg/dL: 17.1% Cumulative blood Pb: Not reported Time-weighted cumulative blood Pb: Not reported	β from linear regression Inhibin B Concurrent blood Pb: 0.40 (p-value 0.40) Cumulative blood Pb: 0.05 (p-value 0.02) Time-weighted cumulative blood Pb: 1.33 (p-value 0.007) Pearson's correlations analysis detected no correlations between concurrent blood Pb levels and FSH, LH, or testosterone. Cumulative blood Pb levels were correlated with FSH and LH, but not testosterone. Time-weighted cumulative blood Pb levels were correlated with LH, but not FSH or testosterone.	LH, FSH, testosterone, age, smoking status, alcohol use, BMI

Table 4-44 (Continued): Summary of recent epidemiologic studies of associations between Pb exposure indicators and hormones for males.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Data for Pb Biomarker or other Exposure Indicator	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Naha and Manna (2007)	FSH, LH, testosterone	Non-occupationally exposed controls and occupationally exposed workers $N_{controls}$=50 $N_{low\ exposure}$=30 $N_{high\ exposure}$=20 Bangalore, India NS	Occupational cohort study using ANOVA, Student's t-test, and Scheffe's F-test	Categorized by work history as controls, low exposure (7-10 yr of exposure for 8 h/day) and high exposure (>10 yr of exposure for 8 h/day) Mean (SD) Blood Pb measurement Controls 10.25 (2.26) Low exposure 50.29 (3.45) High exposure 68.26 (2.49) Semen Pb measurement Controls 2.99 (0.76) Low exposure 15.85 (1.95) High exposure 25.30 (2.28)	Mean FSH (SD) Control: 2.69 (1.22) Low exposure: 2.58 (1.94) High exposure: 2.16 (0.99) p-values for difference >0.05 Mean LH (SD) Control: 5.14 (2.35) Low exposure: 4.27 (2.52) High exposure: 3.9 (1.69) p-values for difference >0.05 Mean Testosterone (SD) Control: 5.24 (2.40) Low exposure:4.83 (1.21) High exposure: 4.59 (1.27) p-values for difference >0.05	None

[a]Studies are presented in order of first appearance in the text of this section, which is based on study design and methodological strength.

Hormone levels were measured in a few recent epidemiologic studies. In a study of men non-occupationally exposed to Pb in Croatia, increased blood Pb level was associated with increasing serum testosterone and estradiol but decreasing serum prolactin (Telisman et al., 2007). In addition, the analysis of an interaction term for blood Pb and blood Cd levels demonstrated a synergistic effect on increasing serum testosterone levels. No association was observed between blood Pb and FSH or LH. This study controlled for multiple potential confounders, including other metals. Among men recruited from infertility clinics in Michigan, median blood Pb levels were much lower than those observed in the other studies of Pb and hormone levels among men (Meeker et al., 2010). No association was detected between blood Pb and levels of FSH, LH, inhibin B, sex hormone-binding globulin (SHBG), free androgen index (FAI), or a measure of Leydig cell function (testosterone/LH). A positive association between the highest quartile of blood Pb and testosterone was present, but this association did not persist when other metals were included in the model. Similarly, another study of men recruited from infertility clinics observed no association between Pb concentrations from seminal plasma, whole blood, or blood plasma and FSH, LH, or testosterone (Mendiola et al., 2011).

A study of occupationally-exposed men in Taiwan reported an association between measures of cumulative blood Pb levels and inhibin B levels, but no association was detected with concurrent blood Pb levels (Hsieh et al., 2009a). A correlation between cumulative blood Pb measures and LH levels was detected but correlations were not present with FSH or testosterone levels. No correlations were apparent between FSH, LH, or testosterone and current blood Pb levels. Another occupational study of men with high blood Pb levels reported no difference in serum FSH, LH, and testosterone among the three groups (controls: mean blood Pb 10.25 µg/dL, low exposure: mean blood Pb 50.29 µg/dL, high exposure: mean blood Pb 68.26 µg/dL) (Naha and Manna, 2007). This study did not assess any potential confounding factors.

In a recent toxicological study, Rubio et al. (2006) observed a decrease in testosterone levels in Pb acetate-treated rats in an exposure concentration-related fashion (8-24 mg/kg body weight), and this decrease correlated with reduced lengths of spermatogenic cycle stages VII-VIII (spermiation) and IX-XI (onset of spermatogenesis). Anjum et al. (2010), who dosed 50 day old male rats with 273 or 819 mg/L Pb acetate in drinking water (500 or 1,500 ppm, respectively; blood Pb not reported), found significant decreases in serum testosterone and testicular 3β-HSD and 17β-HSD levels in Pb-exposed animals versus controls. Pandya et al. (2010) reported altered hepatic steroidogenic enzyme activity. Pillai et al. (2012) found gestational and lactational treatment with Pb acetate in Charles Foster rats (subcutaneous injection of 0.05 mg/kg BW/day, blood Pb not reported) induced significant decreases in testicular 17β-HSD and serum testosterone. Biswas and

Ghosh (2006) reported a Pb-induced decrease in serum testosterone and gonadotropins (FSH, LH) with inhibition of spermatogenesis, however, there was a statistically significant increase in the adrenal steroidogenic enzyme, Δ5-3β-HSD, activity and serum corticosterone levels indicating disruption of the adrenocortical process. Exposure concentration-dependent decreases in serum testosterone were reported in Pb-exposed male rats (Anjum et al., 2010). In contrast, Salawu et al. (2009) did not observe a decrease in serum testosterone between control animals and animals administered 10,000 ppm Pb acetate in drinking water for 8 weeks. Allouche et al. (2009) not only did not observe any statistically significant changes in serum FSH or LH, but reported an increase in serum testosterone levels after 500–3,000 ppm Pb acetate treatment in drinking water (only statistically significant in animals administered 500 ppm Pb acetate). The results of these recent studies further support the theory that compensatory mechanisms in the hypothalamic-pituitary-gonadal axis may allow for the adaptation of exposed animals to the toxic endocrine effects of Pb (Rubio et al., 2006; U.S. EPA, 2006b).

Overall, recent epidemiologic and toxicological studies report mixed findings regarding hormone aberrations in males associated with Pb exposure or Pb biomarker levels. These results are similar to those from the 2006 Pb AQCD (U.S. EPA, 2006b) on the effects of Pb exposure on circulating testosterone levels. Epidemiologic studies are limited by their sample populations, often occupational cohorts or men at infertility clinics, which may not be generalizable. Occupational cohorts may have other exposures that confound the associations, and studies at infertility clinics are subject to selection bias. A few of the recent epidemiologic studies include important confounding factors, such as smoking, but other factors, such as exposure to other metals, were often absent. Additionally, most studies examine concurrent Pb biomarker and hormone levels which may not reflect changes resulting from long-term exposures, as demonstrated by the longitudinal occupational cohort study. Further, in cross-sectional studies, the temporality of effects cannot be established.

4.8.3.3 Effects on Fertility

Epidemiologic studies have been performed comparing Pb and infertility in men. The SMART study is a longitudinal study that examined the success of in vitro fertilization (IVF) treatment for women and their partners starting their first round of treatment (Bloom et al., 2011b; Bloom et al., 2010). A small number of the male partners participated (n=16). Their mean (SD) blood Pb level was 1.50 (0.80) µg/dL. Higher blood Pb levels, measured at the time of oocyte retrieval in female partners, were associated with greater oocyte fertilization (OR 1.08 [95% CI: 0.97, 1.21] per 1 µg/dL increase in

blood Pb when adjusted for Cd, Hg, age, cigarette smoking, race/ethnicity, and creatinine), which is not the expected direction (Bloom et al., 2010). However, higher blood Pb was associated with lower embryo cell number, a predictor of IVF success, and with higher embryo fragmentation score, an inverse predictor of IVF success (OR for embryo cell number: 0.58 [95% CI: 0.37, 0.91]; OR for embryo fragmentation score: 1.47 [95% CI: 1.11, 1.94] per 1 µg/dL, controlled for age, race/ethnicity, cigarette smoking, creatinine, Cd, and Hg, plus day of embryo transfer for embryo cell number) (Bloom et al., 2011b). A case-control study conducted in Turkey reported that concurrent blood and seminal plasma Pb levels were different in fertile (n=45; mean [SD] blood Pb: 23.16 [5.59] µg/dL) and infertile men (n=50; mean [SD] blood Pb: 36.82 [12.30] µg/dL) ($p < 0.001$, ANOVA) (Kiziler et al., 2007). There was no control for potential confounding factors although the relationship persisted when limited to non-smokers. Another case-control study examined occupational Pb exposure (determined by self-report of occupational exposure in the past month) and detected no difference in reported exposure for infertile (n=650) versus fertile men (n=698) (unadjusted OR 0.95 [95% CI: 0.6, 1.6]) (Gracia et al., 2005). Blood Pb was not measured but approximately 5.0% of infertile men and 5.3% fertile men reported occupational exposure to Pb. A limitation present in these studies is that the cases included are men who are seeking help at fertility clinics; the study populations are not a sample of the general population regarding fertility. The results could be biased due to the recruitment of individuals going to an infertility clinic, who may be different than individuals suffering from infertility without knowing it or without going to a clinic.

Recent animal toxicology studies assessed paternal-mediated reproductive fitness by examining the reproductive success of Pb-exposed males with non-exposed control females. Anjum et al. (2010) found that adult male rats that were exposed to 273 or 819 mg/L Pb acetate in drinking water (500 or 1,500 ppm, respectively; blood Pb not reported) spent a significantly longer time copulating than did their control littermates. The Pb-exposed males were less successful copulators with only 73% of the 500 ppm Pb acetate exposed males, and 53% of the 1,500 ppm exposed males generating copulatory plugs in the unexposed female mates. While the number of pregnant females did not significantly differ from controls, Pb exposed males contributed to the formation of significantly fewer implantations/dam, and significantly fewer fetuses/dam. Pb-exposed males were able to sire offspring, but produced fewer offspring per litter. In a group of males rats with co-exposure to Pb and the herb Centella asiatica, these reproductive decrements were attenuated relative to rats exposed to Pb alone (adult albino male rats, 1,500 ppm Pb acetate in drinking water for 70 days) (Sainath et al., 2011).

Overall, large, well-conducted epidemiologic studies of Pb exposure and fertility in males are lacking. The few available studies reported inconsistent findings. Toxicological studies demonstrated paternal associated subfecundity (fewer pups sired per pregnancy) with altered mating behavior (longer time spent copulating), albeit in studies with no blood Pb levels reported. Supplementation with antioxidants in a separate study showed restoration of this subfecundity, possibly contributing mode of action support to this Pb-induced decreased fertility in male rodents.

4.8.3.4 Effects on Morphology and Histology of Male Sex Organs

Recent toxicological studies further support historical findings that showed an association between Pb exposure and changes in the sex organs as well as germ cells. Histological changes in testes of Pb nitrate-treated adult animals (a single i.p. dose of 12.5, 25, or 50 mg/kg of BW and sacrifice 48 hours later) included seminiferous tubule atrophy, Sertoli cell and Leydig cell shrinkage with pyknotic nuclei (Shan et al., 2009; Wang et al., 2006a), dilatation of blood capillaries in the interstitium, undulation of basal membrane, and occurrence of empty spaces in seminiferous epithelium (adult male Wistar rats, single i.p. dose of 12, 25 or 50 mg/kg BW Pb acetate) (Massanyi et al., 2007). Pillai et al. (2010) found gestational and lactational treatment with Pb acetate in Charles Foster rats (subcutaneous injection of 0.05 mg/kg BW/day) induced significant decreases in absolute organ weight (testes and epididymis) and significant decreases in relative epididymal weight. Anjum et al. (2010), who exposed 50 day old male albino Wistar/NIN rats to Pb acetate (273 or 819 mg/L in drinking water, 500 or 1,500 ppm, respectively, blood Pb levels not reported) for 45 days, reported significant decreases in relative reproductive organ weight (epididymis, testis, vas deferens, and seminal vesicle) in Pb-exposed animals.

4.8.3.5 Summary of Effects on Male Reproductive Function

Evidence of associations between Pb exposure and male reproductive function varies by outcome. The strongest evidence of an association is the relationship observed between Pb and negative effects on sperm and semen in both recent epidemiologic and toxicological studies and studies reviewed in previous Pb AQCDs. Decrements in sperm count, sperm production rate and semen quality were reported in animal toxicological studies in rodents with drinking water Pb exposure [(Sokol and Berman, 1991; Sokol et al., 1985), blood Pb level 34-37 µg/dL] and rabbits treated subcutaneously with Pb [(Moorman et al., 1998), blood Pb levels of 25 µg/dL]. Rodents exposed to Pb had direct effects of Pb on sperm DNA, i.e., elevated levels of DNA damage [(Nava-Hernandez et

al., 2009), blood Pb levels 19 and 22 µg/dL]. Histological or ultrastructural damage to the male reproductive organs was reported in studies of rodents [(El Shafai et al., 2011), blood Pb level 5.1 µg/dL] and non-human primates [(Singh et al., 1993a), blood Pb level 43 µg/dL]. Subfecundity has been reported in unexposed females mated to Pb exposed males (decreased number of pups born/litter). Also, sperm from Pb-exposed rats (blood Pb 33 to 46 µg/dL) used for in vitro fertilization of eggs harvested from unexposed females yielded lower rates of fertilization (Sokol et al., 1994). Many of the epidemiologic studies included occupational cohorts, which had high blood Pb levels (≥ 25µg/dL), or men attending infertility clinics, which have potential selection bias. Additionally, control for potential confounding factors, such as other workplace exposures, was not often performed. A study of men (who were attending a clinic for an infertility exam or to donate semen for use in artificial insemination) did control for multiple factors, including other metals and smoking status, and reported an association between blood Pb levels and some indicators of poor sperm quality (Telisman et al., 2007). Recent toxicological studies also reported an association between Pb exposure and decreases in reproductive organ weight, organ histological changes in the testes and germ cells. Male rats exposed to Pb also showed subfecundity, in that they produced smaller litters when mated with unexposed females (Anjum et al., 2010). Further coherence for these findings in laboratory animal models is found with findings in the ecological literature for the effects of Pb exposure on reduced fecundity in terrestrial and aquatic animal species (Sections 6.4.5.2, 6.3.4.2, and 6.4.5.3). Similar to the 2006 Pb AQCD (U.S. EPA, 2006b), recent epidemiologic and toxicological studies reported inconsistent results regarding hormone aberrations associated with Pb exposure. Mixed findings were also apparent among epidemiologic studies of fertility among men.

4.8.4 Effects on Female Reproductive Function

The epidemiologic studies on Pb and female reproductive function presented in the 2006 Pb AQCD (U.S. EPA, 2006b) provided little evidence for an association between Pb biomarkers and effects on female reproduction and fertility. However, the 1986 and 2006 Pb AQCDs (U.S. EPA, 2006b, 1986a) reported toxicological findings that Pb exposure was associated with effects on female reproductive function that can be classified as alterations in female sexual maturation, effects on fertility and menstrual cycle, endocrine disruption, and changes in morphology or histology of female reproductive organs including the placenta. Since the 2006 Pb AQCD, many epidemiologic studies have been published regarding Pb biomarker levels in women and reproductive effects. The studies presented in the following text and tables are grouped

by study design and methodological strength. In addition, recent toxicological studies add further knowledge of Pb-related effects on the female reproductive system.

4.8.4.1 Effects on Female Sex Endocrine System and Estrus Cycle

Multiple epidemiologic studies have examined the association between blood Pb levels and hormone levels and the estrus cycle. Recent epidemiologic studies (characterized in Table 4-45) were cross-sectional in design, analyzing measures of Pb and hormones that were collected either concurrently or close in time. These studies support the toxicological findings, which are the major body of evidence on endocrine effects of Pb.

Table 4-45 Summary of recent epidemiologic studies of associations between blood Pb levels and hormones for females.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Blood Pb Mean (SD) in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Krieg (2007)	FSH, LH	Women aged 35-60 yr from the NHANES III study N=3,375 U.S. 1988-1994	Cross-sectional study using linear regression	2.8	Linear regression slope (95% CI) for log-transformed Pb FSH: Post-menopausal 22.2 (13.5, 30.8) Pregnant 0.1 (-0.1, 0.3) Menstruating at time of exam 2.1 (-2.1, 6.3) Both ovaries removed 32.6 (10.1, 55.1) Birth control pills being used -6.3 (-10.0, -2.5) Pre-menopausal 8.3 (3.8, 12.7) LH: Post-menopausal 6.2 (3.0, 9.5) Pregnant -0.8 (-1.9, 0.4) Menstruating at time of exam -0.3 (-1.8, 1.3) Both ovaries removed 10.0 (1.1, 18.9) Birth control pills being used: -0.6 (-2.9, 1.6) Pre-menopausal 1.7 (-0.6, 4.1)	Age, total bone mineral density, serum cotinine, alcohol use, current breast feeding, hysterectomy, one ovary removed, Norplant use, radiation or chemotherapy, hormone pill use, vaginal cream use, hormone patch use

Table 4-45 (Continued): Summary of recent epidemiologic studies of associations between blood Pb levels and hormones for females.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Blood Pb Mean (SD) in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Pollack et al. (2011)	FSH, estradiol, LH, progesterone, and cycle length	Healthy, premenopausal women aged 18-44 yr with menstrual cycle length of 21-35 days, BMI of 18-35 kg/m^2, not recently using birth control, not planning to become pregnant, and not breast feeding N=252 Buffalo, NY 2005-2007	Longitudinal cohort using nonlinear mixed models with harmonic terms and weighted linear mixed models	0.93 IQR: 0.68, 1.20	**Mean % Estradiol** 0.30-0.72 µg/dL: Ref 0.73-1.10 µg/dL: 8.2 (-1.2, 18.6) 1.11-6.20 µg/dL: 4.7 (-4.7, 15.2) **Amplitude Estradiol** 0.30-0.72 µg/dL: Ref 0.73-1.10 µg/dL: -0.01 (-0.06, 0.04) 1.11-6.20 µg/dL: -0.02 (-0.7, 0.03) Phase Shift Estradiol 0.30-0.72 µg/dL: Ref 0.73-1.10 µg/dL: -0.09 (-0.24, 0.05) 1.11-6.20 µg/dL: 0.14 (-0.01, 0.29)	Age, BMI, race Also examined, but did not include: smoking, income, education, physical activity, parity, dietary Fe, fish consumption, shellfish consumption, vegetable consumption, total calories

Table 4-45 (Continued): Summary of recent epidemiologic studies of associations between blood Pb levels and hormones for females.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Blood Pb Mean (SD) in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Pollack et al. (2011) (Continued)	FSH, estradiol, LH, progesterone, and cycle length	Healthy, premenopausal women aged 18-44 yr with menstrual cycle length of 21-35 days, BMI of 18-35 kg/m^2, not recently using birth control, not planning to become pregnant, and not breast feeding N=252 Buffalo, NY 2005-2007	Longitudinal cohort using nonlinear mixed models with harmonic terms and weighted linear mixed models	0.93 IQR: 0.68, 1.20	**Mean % FSH** 0.30-0.72 µg/dL: Ref 0.73-1.10 µg/dL: 8.0 (-0.9, 17.7) 1.11-6.20 µg/dL: 3.6 (-5.3, 13.3) **Amplitude FSH** 0.30-0.72 µg/dL: Ref 0.73-1.10 µg/dL: -0.01 (-0.03, 0.02) 1.11-6.20 µg/dL: -0.02 (-0.04, 0.01) Phase Shift FSH 0.30-0.72 µg/dL: Ref 0.73-1.10 µg/dL: -0.06 (-0.25, 0.12) 1.11-6.20 µg/dL: -0.02 (-0.21, 0.18) **Mean % LH** 0.30-0.72 µg/dL: Ref 0.73-1.10 µg/dL: 5.1 (-5.1, 16.4) 1.11-6.20 µg/dL: -0.5 (-10.5, 10.7) **Amplitude LH** 0.30-0.72 µg/dL: Ref 0.73-1.10 µg/dL: -0.01 (-0.03, 0.02) 1.11-6.20 µg/dL: -0.02 (-0.04, 0.01) **Phase Shift LH** 0.30-0.72 µg/dL: Ref 0.73-1.10 µg/dL: -0.16 (-0.36, 0.03) 1.11-6.20 µg/dL: -0.11 (-0.32, 0.10)	Age, BMI, race Also examined, but did not include: smoking, income, education, physical activity, parity, dietary Fe, fish consumption, shellfish consumption, vegetable consumption, total calories

Table 4-45 (Continued): Summary of recent epidemiologic studies of associations between blood Pb levels and hormones for females.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Blood Pb Mean (SD) in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Pollack et al. (2011) (Continued)	FSH, estradiol, LH, progesterone, and cycle length	Healthy, premenopausal women aged 18-44 yr with menstrual cycle length of 21-35 days, BMI of 18-35 kg/m^2, not recently using birth control, not planning to become pregnant, and not breast feeding N=252 Buffalo, NY 2005-2007	Longitudinal cohort using nonlinear mixed models with harmonic terms and weighted linear mixed models	0.93 IQR: 0.68, 1.20	**Mean % Progesterone** 0.30-0.72 µg/dL: Ref 0.73-1.10 µg/dL: 7.5 (0.1, 15.4) 1.11-6.20 µg/dL: 6.8 (-0.8, 14.9) **Amplitude Progesterone** 0.30-0.72 µg/dL: Ref 0.73-1.10 µg/dL: 0.07 (0.01, 0.15) 1.11-6.20 µg/dL: -0.06 (-0.13, 0.01) Phase Shift Progesterone 0.30-0.72 µg/dL: Ref 0.73-1.10 µg/dL: 0.04 (-0.06, 0.15) 1.11-6.20 µg/dL: 0.15 (0.05, 0.26) Linear models β (95% CI) **Estradiol:** 0.03 (-0.05, 0.11) **FSH:** -0.01 (-0.07, 0.06) **LH:** 0.02 (-0.06, 0.10) **Progesterone:** 0.06 (-0.04, 0.17) **OR** (95% CI) for anovulation per 1 µg/dL: 1.20 (0.62, 2.34)	Age, BMI, race Also examined, but did not include: smoking, income, education, physical activity, parity, dietary Fe, fish consumption, shellfish consumption, vegetable consumption, total calories

Table 4-45 (Continued): Summary of recent epidemiologic studies of associations between blood Pb levels and hormones for females.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Blood Pb Mean (SD) in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Jackson et al. (2011)	FSH, estradiol, LH, progesterone, and cycle length	Healthy, pre-menopausal women aged 18-44 yr with menstrual cycle length of 21-35 days, BMI of 18-35 kg/m^2, not recently using birth control, not planning to become pregnant, and not breast feeding N=252 Buffalo, NY 2005-2007	Longitudinal cohort study using linear regression and logistic regression	Median: 0.87 IQR: 0.68, 1.20	**Adjusted percent change** (95% CI) in serum hormone level for change in blood Pb **FSH**: -2.5 (-11.2, 7.0) **Estradiol:** 4.9 (-5.0, 15.9) **LH:** 2.5 (-12.3, 19.9) **Progesterone**: 4.6 (-12.2, 24.6) **Cycle length**: 0.2 (-2.8, 3.3) **OR** (95% CI) per unit Pb <25 day vs. 25-35 day cycle length: 0.9 (0.4, 2.3) >35 day vs. 25-35 day cycle length: 0.5 (0.1, 1.9)	Cd, Hg, age, race /ethnicity
Chang et al. (2006)	Estradiol	Women receiving care at a infertility clinic in 2000-2001 or delivering a normal infant at a nearby medical center in 1999 N=147 Kaohsiung City, Taiwan 1999, 2000-2001	Case-control study using multivariate linear regression	3.12 (0.19)	Linear regression β (SE) per 1 µg/dL Pb 1.18 (0.60) p-value: 0.049	Not specified

[a]Studies are presented in order of first appearance in the text of this section, which is based on study design and methodological strength.

An epidemiologic study using the NHANES III data and including women aged 35-60 years old examined the relationship between blood Pb levels (mean 2.8 µg/dL) and serum FSH and LH (Krieg, 2007). Deviation from normal FSH and LH levels may indicate endocrine disruption related to ovary functioning. Researchers found that higher blood Pb levels were associated with higher levels of serum FSH and LH among both postmenopausal women and women with both ovaries removed. There was also a trend of increasing serum FSH with blood Pb levels for pre-menopausal women who were not menstruating at the time of the exam or pregnant, although the association was not statistically significant for LH. A limitation of this portion of the study is that FSH and LH were measured without attention to day of a woman's menstrual cycle and LH and FSH are known to vary throughout the cycle of non-menopausal, cycling women who are not taking birth control pills. Higher blood Pb levels were associated with lower levels of serum FSH among women taking birth control pills. The inverse association was also present for LH, but it was not statistically significant. No associations between blood Pb and FSH or LH were apparent for women who were menstruating at the time of the exam or were pregnant. Further analysis indicated that the lowest level of blood Pb for which a statistically significant association between blood Pb and FSH could be observed was 1.7 µg/dL among women with their ovaries removed. For LH, the lowest level of blood Pb for which a statistically significant association between blood Pb and LH could be observed was 2.8 µg/dL among postmenopausal women. Associations between hormones and blood Pb level were also investigated using the BioCycle study cohort (Jackson et al., 2011; Pollack et al., 2011). These women were premenopausal with normal cycles and not on birth control. Blood Pb was measured at enrollment and hormones were measured multiple times throughout the menstrual cycle. Neither study detected an association between unit change in blood Pb and hormone levels. However, in examination of tertiles of Pb, women in the highest tertile blood Pb (1.11-6.20 µg/dL) had higher mean progesterone and longer length of a phase shift compared to women in the lowest tertile (0.30-0.72 µg/dL) (Pollack et al., 2011). Other associations were observed but were not statistically significant (Pollack et al., 2011). No associations were detected for anovulation (Pollack et al., 2011) or for cycle length (Jackson et al., 2011). Another epidemiologic study was performed in Kaohsiung City, Taiwan among two groups of women aged 23-44 years: those who were seeking help at a fertility clinic after one year of trying to conceive, and those who had previously delivered an infant and were identified from medical records of a postpartum care unit (Chang et al., 2006). The mean (SD) concurrent blood Pb level in this study was 3.12 (0.19) µg/dL. The study reported a positive association between blood Pb levels and serum estradiol concentrations during the early follicular phase, which reflects ovary activity.

The effect of Pb exposure on the female endocrine system was demonstrated in toxicological studies reviewed in the 1986 and 2006 Pb AQCDs (U.S. EPA, 2006b,

1986a). However, the mechanism by which Pb affects the endocrine system has not been fully elucidated. Several recent articles continue to demonstrate that Pb alters the concentration of circulating hormones in female experimental animals. As mentioned in the 2006 Pb AQCD, Pine et al. (2006) observed that maternal Pb exposure (during gestation and lactation) caused a decrease in basal LH levels in pre-pubertal female Fischer 344 rat pups as compared to control, non-Pb-exposed pups. Dumitrescu et al. (2008a) observed an alteration of hormone levels in adult female Wistar rats that ingested Pb acetate (50, 100, 150 ppb) in drinking water for 6 months; measurements were made during the pro-estrous stage of the estrous cycle to allow for consistent timing and control for cyclic hormonal variation. The authors reported decreases in FSH, estradiol, and progesterone levels with increases in LH and testosterone levels. Nampoothiri and Gupta (2008) administered Pb acetate to Charles Foster female rats 5 days before mating and during the gestational period at a concentration that did not affect reproductive performance, implantation or pregnancy outcome (0.05 mg/kg body weight). They observed a decrease in steroidogenic enzymes, 3β- hydroxysteroid dehydrogenase (HSD) and 17β-HSD, activity in reproductive organs, as well as a decrease in steroid hormones (progesterone and estradiol), suggesting that chronic exposure to low levels of Pb may affect reproductive function of mothers and their offspring. Similarly, Pillai et al. (2010) reported impaired ovarian steroidogenesis in Charles Foster adult female rats (PND56) from dams treated gestationally and lactationally to Pb acetate (subcutaneous daily injections of 0.05 µg/kg BW). Pillai observed a decrease in steroidogenic enzymes, 3β-HSD and 17β-HSD, but saw no changes in ovarian steroidogenic acute regulatory protein (StAR) or CYP11 mRNA levels indicating Pb-induced inhibition of ovarian steroidogenesis.

Kolesarova et al. (2010) conducted an in vitro study to examine the secretory activity of porcine ovarian granulose cells after Pb administration for 18 hours. The results of the study showed that Pb acetate concentrations of 0.046 mg/mL and 0.063 mg/mL statistically significantly inhibited insulin-like growth factor-1 (IGF-1) release, but concentrations of 0.25 mg/mL and 0.5 mg/mL Pb did not influence IGF-1 release. Progesterone release was not affected by Pb treatment; however, Pb caused a reduction in LH and FSH binding in granulose cells and increased apoptosis as evidenced by increased expression of caspase-3 and cyclin B1, suggesting a Pb-induced alteration in the pathways of proliferation and apoptosis of porcine ovarian granulose cells. Decreased gonadotropin binding was also observed in rats after Pb subcutaneously administration (0.05 mg/kg body weight daily) before mating and during pregnancy, resulting in a blood Pb of 2.49 µg/mL (Nampoothiri and Gupta, 2006).

No recent toxicological studies were found that examined Pb-induced effects on the estrus cycle.

Overall, toxicological studies report alterations in hormone levels related to blood Pb concentration. Similarly, epidemiologic studies reported associations between concurrent/closely timed blood Pb levels and hormone levels in female adults. Although Pb-associated changes in hormone levels are observed, there are discrepancies and the hormones examined vary by study. One explanation for the inconsistent findings is that changes could vary based on current hormonal and reproductive status of the participants. Also, the covariates included in statistical models as potential confounders varied among studies, which could contribute to between study heterogeneity. Another limitation of the epidemiologic studies is that not all of the studies investigated important confounders, such as other metal exposures or smoking. Additionally, the cross-sectional design of these studies leaves uncertainty regarding Pb exposure magnitude, timing, duration, and frequency that contributed to the observed associations.

4.8.4.2 Effects on Fertility

Previous studies indicated that Pb exposure does not produce total sterility, but it can disrupt female fertility (U.S. EPA, 2006b). Recent epidemiologic studies and studies in experimental animals have inconsistent results. The recent epidemiologic studies are summarized in Table 4-46. Most of these studies examined biological measures of Pb collected at or during the period of possible fertilization or start of fertility treatment, although Bloom et al. (2011a) measured blood Pb at baseline and followed women for at least 12 menstrual cycles (or until pregnancy).

Table 4-46 Summary of recent epidemiologic studies of associations between Pb biomarker levels and fertility for females.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Pb Biomarker Mean (SD) in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Bloom et al. (2011a)	Achieving pregnancy	Women who were aged 18-34 yr, were previously part of a study about fish consumption, and were not currently pregnant and were followed for 12 menstrual cycles or until pregnant N=80 New York 1996-1997	Longitudinal cohort using Cox proportional hazards	Blood Pb at baseline No positive pregnancy test: 1.55 (0.16) Positive pregnancy test: 1.54 (0.12)	Probability (95% CI) for achieving pregnancy per 0.6 µg/dL blood Pb: -0.031 (-1.066, 1.004)	Baseline As, baseline Cd, baseline Mg, baseline Ni, baseline Se, baseline Zn, total serum lipids, age, parity, frequency of intercourse during fertility window, alcohol use, cigarette use
Chang et al. (2006)	Infertility	Women receiving care at a infertility clinic in 2000-2001 or delivering a normal infant at a nearby medical center in 1999 Cases: N=64 Controls: N=83 Kaohsiung City, Taiwan 1999, 2000-2001	Case-control study using unconditional logistic regression	Blood Pb 3.12 (0.19)	OR (95% CI) for Infertility ≤ 2.5 µg/dL: 1.00 (Ref) >2.5 µg/dL: 2.94 (1.18, 7.34)	Age, BMI, active smoking, use of Western medicine Considered but did not include: irregular menstruation, age at first menses, marital status, passive smoking, contraceptive drugs

Table 4-46 (Continued): Summary of recent epidemiologic studies of associations between Pb biomarker levels and fertility for females.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Pb Biomarker Mean (SD) in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Bloom et al. (2010)	Oocyte maturity, oocyte fertilization	Women who were part of the Study of Metals and Assisted Reproductive Technologies (SMART): women referred to the Center for Reproductive Health of UCSF for infertility treatment and their first IVF procedure N=15 California 2007-2008	Longitudinal cohort using multivariable log-binomial regression	Blood Pb at the time of oocyte retrieval 0.82 (0.32)	RR per 1 µg/dL **Oocyte maturity** (determined by Metaphase II arrest): 0.54 (0.31, 0.93) 0.25 (0.03, 2.50)* Oocyte fertilization: 0.97 (0.66, 1.43) 1.09 (0.72, 1.65)*	Age, cigarette smoking, race/ethnicity *Also, adjusted for Cd.
Bloom et al. (2011b)	Embryo cell number, embryo fragmentation score	Women who were part of the Study of Metals and Assisted Reproductive Technologies (SMART) (women referred to the Center for Reproductive Health of UCSF for infertility treatment and their first IVF procedure) and who generated embryos N=24 California 2007-2008	Longitudinal cohort using ordinal logistic regression	Blood Pb at the time of oocyte retrieval 0.83 (0.30)	OR per 1 µg/dL Higher embryo cell number: 0.25 (0.07, 0.86) Higher embryo fragmentation score: 1.71 (0.45, 6.56)	Age, race/ethnicity, cigarette smoking, urine creatinine, Hg, Cd Additionally included for embryo cell number: day of embryo transfer

Table 4-46 (Continued): Summary of recent epidemiologic studies of associations between Pb biomarker levels and fertility for females.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Pb Biomarker Mean (SD) in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Al-Saleh et al. (2008a)	Achieving pregnancy and/or fertilization	Women aged 19-50 yr undergoing IVF Pregnancy: N=203 No pregnancy: N=321 Fertility: N=556 No fertility: N=63 Riyadh, Saudi Arabia 2002-2003	Longitudinal cohort using logistic regression	Blood Pb Follicular fluid Pb Blood Pb: 3.34 (2.24) Blood Pb levels >10 µg/dL: 1.7% Follicular fluid: 0.68 (1.82)	OR (95% CI) (unit not given, assume results are per 1 µg/dL) **Pregnancy** Blood Pb: 0.55 (0.23, 1.31) Follicular fluid Pb: 1.36 (0.91, 2.02) **Fertilization** Blood Pb: 0.30 (0.08, 1.03) Follicular fluid Pb: 1.45 (0.69, 3.02) Note: In a reduced adjusted model for fertilization, the OR for blood Pb was 0.38 (0.14, 0.99)	Age, husband's age, BMI, location and duration at that location, previous location and duration at that location, age at first menses, number of days of menstrual cycle, education, work status, husband's education, family income, husband's smoking status, blood and follicular Cd and Hg, follicular cotinine Also included for pregnancy as outcome: coffee consumption, tea consumption, caffeinated soft drink consumption,
Silberstein et al. (2006)	Achieving pregnancy	Women undergoing IVF at the study hospital Pregnancy: N=4 No pregnancy: N=5 Providence, RI NS	Longitudinal cohort study using Mann-Whitney U-test	Follicular fluid Pb Not given quantitatively Estimated from a figure in the paper: Median Pb in follicular fluid of pregnant women: ~1.3 Median Pb in follicular fluid of non-pregnant women: ~2.2	P-value for difference in medians by Mann-Whitney U test: 0.0059 *Note, This study only included 9 women	None

[a]Studies are presented in order of first appearance in the text of this section, which is based on study design and methodological strength.

A prospective cohort study followed women who previously participated in a study of fish consumption for a length of up to 12 menstrual cycles and investigated the relationship between blood Pb levels at baseline and having a positive pregnancy test at some point during the next 12 menstrual cycles (Bloom et al., 2011a). No association was observed between blood Pb and achieving pregnancy.

Among women aged 23-44 years, a difference in blood Pb was reported between women who were seeking help at a fertility clinic after one year of trying to conceive and women who had previously delivered an infant and were identified from medical records of a postpartum care unit at a medical center (Chang et al., 2006). Higher odds of infertility were observed when comparing women with blood Pb levels >2.5 µg/dL to those with blood Pb levels ≤ 2.5 µg/dL although this study is limited by its case-control design.

Epidemiologic studies have also examined women having difficulty conceiving by performing studies among patients of fertility clinics or undergoing IVF. The Study of Metals and Assisted Reproductive Technologies (SMART) enrolled women undergoing their first round of IVF and investigated multiple steps before pregnancy as the outcomes (Bloom et al., 2011b; Bloom et al., 2010). Higher blood Pb levels were associated with lower oocyte maturity although the wide 95% CIs made interpretation of models controlling for Cd difficult. No association was observed between blood Pb and oocyte fertilization (Bloom et al., 2010). In the examination of markers of IVF success, inconsistent results were observed. Embryo cell number was lower in association with higher blood Pb levels but no association was observed for embryo fragmentation score (Bloom et al., 2011b). Another study examining fertility reported on women in Saudi Arabia aged 19-50 years who were undergoing IVF treatment (Al-Saleh et al., 2008a). Women were categorized as having achieved a pregnancy versus not having achieved a pregnancy and achieved fertilization versus not achieving fertilization. The majority of women had follicular Pb levels that were below the limit of detection; whereas less than 2% of women had blood Pb levels below the limit of detection. In addition, less than 2% of women had blood Pb levels that were above 10 µg/dL. Follicular Pb levels were not correlated with the blood Pb. No association was observed between blood or follicular Pb and pregnancy outcomes in either crude or adjusted models. An association was not detected between follicular Pb and fertilization, but higher blood Pb was associated with lower rates of fertilization. Finally, a study that included nine women undergoing IVF treatment in Rhode Island (Silberstein et al., 2006) found that median follicular Pb levels in women who achieved pregnancy were lower than the follicular Pb levels among nonpregnant women.

Overall, these epidemiologic studies examine a variety of fertility-related endpoints and although some studies demonstrate an association between higher Pb biomarker levels and fertility/pregnancy, as a whole the results are inconsistent across studies. One limitation present in most of these studies is that the participants are women who are seeking help for fertility problems. The participants are not samples of the general population and therefore cannot be generalized to all women of childbearing age. This may also have introduced substantial selection bias into the study.

Animal toxicology studies following female fertility looked at various outcomes. Several studies observed a decrease in litter size when females were exposed to Pb before mating or during pregnancy (Dumitrescu et al., 2008b; Iavicoli et al., 2006a; Teijón et al., 2006). Pups in a study by Teijon et al. (2006) receiving 400 ppm Pb acetate in drinking water had blood Pb of 97 µg Pb/dL blood at 1 week post-weaning and 18.2 µg Pb/dL blood at 2 week post-weaning. Dumitrescu et al. (2008b) observed a modification in sex ratio of pups born to dams exposed to Pb before mating and during the entirety of pregnancy. As the dose of Pb increased, the number of females per litter also increased (i.e., 1 male to 0.8 female in non-Pb-exposed group; 1 male to 0.66 female in 50 ppb Pb acetate group; 1 male to 2.25 females in 100 ppb group; and 1 male to 2.5 females in 150 ppb group). These results are not consistent with earlier results of Ronis et al. (1998b), who did not observe differences in sex ratio dams and offspring were exposed only during pregnancy. Thus, Pb exposure in animal studies during or before pregnancy have shown effects on litter size and mixed effects on sex ratio.

Nandi et al. (2010) demonstrated a concentration-dependent decline in viability rate, maturation, fertilization, and cleavage rates of buffalo oocytes cultured in medium containing 1-10 µg/mL Pb acetate (24 hour culture). Karaca and Şimşek (2007) observed an increase in the number of mast cells in ovary tissue after Pb exposure (2,000 µg/mL in drinking water for 6 weeks prior to estrous monitoring then for 1 additional month during which estrous cyclicity was monitored) suggesting that Pb may stimulate an inflammatory response in the ovaries which may contribute to Pb-induced female infertility.

In contrast, Nampoothiri and Gupta (2008) did not observe any statistically significant change in fertility rate or litter size in female rats subcutaneously administered Pb (0.05 mg/kg body weight daily before mating and during pregnancy) with a resulting blood Pb of 2.49 µg/mL. Although reproductive performance was not affected in this study, the authors did report an alteration in implantation enzymes. Cathepsin-D activity decreased and alkaline phosphatase activity increased after Pb exposure.

In summary, recent epidemiologic and toxicological studies on the effect of Pb on fertility outcomes have generated inconsistent results. Most of the epidemiologic studies are limited by their small sample sizes, potential selection bias, and lack of generalizability due to a focus on women seeking help for infertility. Most of the studies control for multiple potential confounders, such as smoking status and age. The studies from the toxicological literature show that Pb exposure to female experimental animals affects litter size (decreased litter size), sex ratio (ratio of male to female offspring in a litter) and ovarian viability, albeit often at higher doses of Pb than relevant to this ISA. However, the bulk of the toxicological evidence including the current and historical Pb literature (U.S. EPA, 2006b) indicates that increased Pb exposure may decrease female fertility.

4.8.4.3 Ovaries, Embryo Development, Placental function, and Spontaneous Abortions

The 2006 Pb AQCD included studies of Pb exposure among men and women and their associations with spontaneous abortions. The 2006 Pb AQCD concluded that overall there was little evidence to support an association between Pb exposure among women and spontaneous abortion (U.S. EPA, 2006b). Most of the studies examined in the 2006 Pb AQCD assigned exposure based on living near a smelter or working in occupations that often result in Pb exposure and the results of these studies were inconsistent. Little evidence was available in the 2006 Pb AQCD to suggest an association with paternal Pb biomarker levels, and no recent studies have been performed to examine paternal Pb biomarker levels and spontaneous abortion. Since the 2006 Pb AQCD, multiple epidemiologic studies have been published that examine Pb biomarker levels in women and their possible association with spontaneous abortion. Table 4-47 provides information on these longitudinal and cross-sectional studies. Additionally, toxicological studies have studied the effects of Pb on fetal loss and the contribution of the ovaries and placenta to fetal loss.

Table 4-47 Summary of recent epidemiologic studies of associations between Pb biomarker levels and spontaneous abortions.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Pb Biomarker Mean (SD) in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Vigeh et al. (2010)	Pregnancy ended before 20 weeks of gestation	Women who were non-smokers, non-obese, had no chronic health conditions, had their last menstrual period less than 12 weeks prior, and were pregnant with a singleton infant ≥ 1 spontaneous abortions: N=15 No spontaneous abortions: N=336 Tehran, Iran 2006-2008	Longitudinal cohort study using t-test and logistic regression	Maternal blood Pb during weeks 8-12 of pregnancy 3.8 (2.0) Spontaneous abortion: 3.51 (1.42) Non-spontaneous abortion: 3.83 (1.99)	T-test for difference in mean values: p-value 0.41 OR: 0.331 (95% CI: 0.011, 10.096) for an increase in log-transformed blood Pb (assume per 1 µg/dL)	Age, parity, hematocrit, passive cigarette smoking exposure

Table 4-47 (Continued): Summary of recent epidemiologic studies of associations between Pb biomarker levels and spontaneous abortions.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Pb Biomarker Mean (SD) in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Yin et al. (2008)	Anembryonic pregnancy	Women age 25-35 yr and at 8-12 weeks of gestation at study entry; cases were anembryonic pregnancies and controls were normal pregnancies that ended in a live birth between 37-42 weeks Cases: N=40 Controls: N=40 Shanxi Province, China 2004-2006	Case-control study using t-test	Maternal blood Pb after miscarriage for cases and at study enrollment for controls Cases: 5.3 (95% CI: 5.2, 5.9) Controls: 4.5 (95% CI: 3.7, 5.0)	Comparisons between log-transformed blood Pb levels of cases and controls performed via Student's t-test had a p-value of 0.03	None
Lamadrid-Figueroa et al. (2007)	Previous miscarriage	Women who had a previous pregnancy and were currently pregnant with gestational age of ≤ 14 weeks ≥ 1 previous miscarriages: N=71 No previous miscarriages: N=136 Mexico City, Mexico 1997-1999, 2001-2004	Cross-sectional study using Poisson regression	Maternal and umbilical cord blood Pb, maternal bone Pb Overall: Blood Pb: 6.2 (4.5) Plasma Pb: 0.014 (0.013) Cases: Blood Pb: 5.8 (3.4) Plasma Pb: 0.014 (0.013) Controls: Blood Pb: 6.5 (4.9) Plasma Pb: 0.013 (0.013)	**IRR** (95%CI) Categorized Plasma Blood Pb ratio: 1st tertile: 1.00 (Ref) 2nd tertile: 1.16 (p-value 0.61) 3rd tertile: 1.90 (p-value 0.015) **IRR** (95%CI) Per 1 SD increase: Plasma Pb: 1.12 (p-value 0.22) Blood Pb: 0.93 (p-value 0.56) Plasma/Blood Pb ratio: 1.18 (p-value 0.02) Patella Pb: 1.15 (p-value 0.39) Tibia Pb: 1.07 (p-value 0.56)	Age, education

Table 4-47 (Continued): Summary of recent epidemiologic studies of associations between Pb biomarker levels and spontaneous abortions.

Reference[a]	Outcome	Study Population Study Location Years	Methodological Details	Pb Biomarker Mean (SD) in µg/dL	Adjusted Effect Estimates	Potential Confounders Adjusted for in Analysis
Gundacker et al. (2010)	Previous miscarriage	Women recruited during the second trimester of pregnancy ≥ 1 previous miscarriages: N=8 No previous miscarriages: N=22 Vienna, Austria 2005	Cross-sectional study using non-parametric tests	Whole placentas shortly after birth Median (IQR): 25.8 (21.0, 36.8)	Median Placenta Pb: Women who had not previously miscarried: 27 µg/kg Women who had previously miscarried: 39 µg/kg (p-value for difference: 0.039)	None

[a]Studies are presented in order of first appearance in the text of this section, which is based on study design and methodological strength.

A longitudinal study examining spontaneous abortions occurring early in the pregnancy was conducted in Iran (Vigeh et al., 2010). Mean blood Pb concentrations, measured at 8-12 weeks of pregnancy, were similar in women who did and did not have spontaneous abortions. Higher blood Pb levels were not associated with greater odds of spontaneous abortions before 20 weeks of pregnancy. Yin et al. (2008) performed a study in the Shanxi Province of China to examine if plasma Pb levels were associated with anembryonic pregnancies (spontaneous abortions during the first trimester, which account for 15% of all spontaneous abortions). Women were enrolled at 8-12 weeks of gestation. Women who delivered a term pregnancy had mean plasma Pb levels that were lower than those of women who had an anembryonic pregnancy (plasma Pb measured at the time of miscarriage for cases and at 8-12 weeks for controls). Of note, among cases plasma Pb level was inversely correlated with folate and vitamin B12, but this correlation was not observed among those who delivered at term; no models examining plasma Pb levels were adjusted for nutrient status. A study in Mexico City examined a group of pregnant women (maximum gestational period at enrollment was 14 weeks) who had previously been pregnant and either given birth or had a spontaneous abortion (Lamadrid-Figueroa et al., 2007). Women in the highest tertile of plasma/blood Pb ratio had a higher rate of previous spontaneous abortions than did women in the lowest tertile. The authors state that the plasma/whole blood ratio represents bioavailable Pb, which is capable of crossing the placental barrier for a given blood concentration. No association was observed for the relationship between Pb and spontaneous abortions using whole blood, plasma, or bone Pb alone. Similarly, a study of placental Pb levels among pregnant women in Austria observed higher placenta Pb levels among women who had miscarried a previous pregnancy compared to women who had not miscarried a previous pregnancy (Gundacker et al., 2010). It is important to note that the number of women included in the study was small (only 8 women reported previously having a miscarriage)

In toxicological studies, isolated bovine embryo cultures are often used to understand the mechanisms responsible for aberrant embryo development as it may contribute to teratogenesis, fetal loss or negative postnatal pup outcomes. Nandi et al. (2010) demonstrated an exposure concentration-dependent decline in embryo development of fertilized buffalo oocytes cultured for 24 hours in medium containing 0.05-10 µg/mL Pb acetate as evidenced by reduced morula/blastocyst yield and increased four-to eight-cell arrest, embryo degeneration, and asynchronous division. This study provides evidence of the negative effect of Pb on embryo development and contributes mechanistic understanding to Pb-induced pregnancy loss.

A possible explanation for reduced fertility and impaired female reproductive success as a result of Pb exposure is changes in morphology or histology in female sex organs and the placenta (Dumitrescu et al., 2007; U.S. EPA, 2006b). Wang et al. (2009e) observed

that elevated maternal blood Pb (0.6-1.74 µM, ~12.4-36.0 µg/dL) compared to control (0.04 µM, ~0.83 µg/dL) was associated with decreased fetal body weight, pup body length, and placental weight in Wistar rats. The authors reported that placentae from Pb-exposed groups showed concentration-dependent increasing pathology of cytoarchitecture and cytoplasmic organelles. The authors also reported positive expression of NF-κB, a transcription factor that controls the expression of genes involved in immune responses, apoptosis, and cell cycle, in the cytotrophoblasts, decidual cells, and small vascular endothelial cells in rat placenta under a low-level Pb exposure condition which correlated with low blood Pb levels.

Pb-exposed (273 mg/L or 819 mg/L in drinking water, 500 or 1,500 ppm Pb acetate, respectively) male rats from Anjum et al. (2010) that had an exposure concentration-dependent decrease in serum testosterone, decreased male reproductive organ weight and decreased sperm were mated to untreated females. These untreated dams bred to the Pb exposed males concentration-dependent decreased implantation rate and higher pre- and post-implantation loss, indicating paternally mediated fetal loss. The magnitude of these effects in dams was dependent on the concentration of Pb exposure in their male mating partners.

As observed in sperm cells, Pb stimulates changes in antioxidant enzyme activity in rat ovaries indicating that oxidative stress may be a contributing factor in Pb-induced ovarian dysfunction. Nampoothiri et al. (2007) observed a reduction in SOD activity and an increase in CAT activity along with a decrease in glutathione content and an increase in lipid peroxidation in rat granulosa cells after 15 days of Pb treatment (subcutaneously administered Pb, 0.05 mg/kg body weight daily before mating and during pregnancy with a resulting blood Pb of 2.49 µg/mL).

Previous studies demonstrated that Pb can be found in the ovaries and causes histological changes, thus potentially contributing to Pb-induced effects on female fertility (U.S. EPA, 2006b). In support of historical studies, recent studies demonstrate Pb-induced histological changes in ovarian cells of pigs (Kolesarova et al., 2010) and rats (Nampoothiri et al., 2007; Nampoothiri and Gupta, 2006). Kolesarova et al. (2010) observed a reduction of the monolayer of granulosa cells after Pb addition (0.5 mg/mL, 18 hours culture). Nampoothiri and Gupta (2006) reported that Pb treatment caused a decrease in cholesterol and total phospholipid content in the membranes of granulosa cells which resulted in increased membrane fluidity (subcutaneously administered Pb, 0.05 mg/kg body weight daily before mating and during pregnancy with a resulting blood Pb of 2.49 µg/mL).

Overall, the recent studies support the conclusions of the 2006 Pb AQCD (U.S. EPA, 2006b) that there is mixed evidence among epidemiologic studies to suggest an

association between Pb and spontaneous abortions. It is important to note that studies of spontaneous abortions are difficult to conduct. The majority of spontaneous abortions occur during the first trimester, which makes them difficult to capture. Women may miscarry before being enrolled in a study and many women may not have known they were pregnant when they miscarried. This limits the ability to detect subtle effects, especially if higher Pb exposures do lead to increased risk of early spontaneous abortions. In addition, some studies are limited by their retrospective examination of current Pb biomarker levels in relation to previous miscarriages. Sample size is another limitation of the available epidemiologic studies. The epidemiologic studies also had little control for potential confounding factors, with some studies including no potential confounders in their analyses. Toxicological data provide a potential mechanistic explanation of the contribution of Pb exposure to spontaneous abortions. These laboratory data show that Pb exposure impaired placental function, induced oxidative stress and histological changes in the ovaries, and affected embryo development. The toxicological and epidemiologic data provide inconsistent findings for the role of Pb in spontaneous abortions.

4.8.4.4 Effects on Breast Milk

Experiments in laboratory animals have shown that dietary manipulation of maternal fatty acid (FA) levels in diet can worsen Pb-related behavioral effects of offspring after lactational Pb exposure (Lim et al., 2005). To determine if components of dam milk contributed to this change, dam milk fatty acids were altered via diet. Diets deficient in (n-3) fatty acids (FA[s]) can lead to a deficiency of DHA, which is essential for proper nervous system development. Lim et al. (2005) found that dam Pb exposure (Long-Evans rats, 2,000 ppm Pb acetate trihydrate/BW) during lactation (PND0-PND21) led to a decrement in non-essential fatty acids in the maternal organs at PND25 but with blood Pb levels much higher than those relevant to this ISA (mean [SD] blood Pb levels in dams: 308 [56] µg/dL). In animals with a diet deficient in (n-3) FAs, there was a Pb-diet interaction with a specific size PUFA (i.e., a 20-carbon [n-6] PUFA). In general, Pb exposure caused a decrement in shorter chain monounsaturated and saturated FAs in maternal organs.

Supplementation of the maternal diet with calcium can be an especially important modulator of Pb mobilization into maternal blood during periods of high calcium demand including pregnancy/lactation. For example, mothers with elevated blood Pb levels given calcium phosphate and ascorbic acid supplementation during pregnancy and lactation had a 90% decrease in placental Pb content and a 15% decrease in the concentration of Pb in breast milk (Altmann et al., 1981) versus the control group that did not receive dietary treatment. Another study (Gulson et al., 2004a) has shown that calcium supplementation

during lactation is less beneficial in modulating maternal blood Pb levels (mean blood Pb at first sampling was 2.4 µg/dL); the Gulson cohort (Gulson et al., 2004a) was limited by power (n=10 women). In a cohort of women from Mexico City, daily calcium supplementation during lactation reduced maternal blood Pb by 15–20% and Pb in breast milk by 5–10% (Ettinger et al., 2004a). Another study by the same investigators showed that using calcium supplements daily during pregnancy also reduced blood Pb levels during pregnancy (Ettinger et al., 2009) with the effect strongest in women with higher biomarkers of Pb exposure (elevated baseline bone Pb or >5 µg/dL blood Pb) or in women with higher Pb exposure (self-reported use of Pb-glazed ceramics). Thus, dietary modulation with calcium supplementation during pregnancy and lactation may decrease the amount of Pb to which the developing fetus of infant is exposed. The evidence for this seems especially strong for protection during pregnancy and more mixed for protective effects of calcium during lactation.

4.8.4.5 Summary of Effects on Female Reproductive Function

In summary, Pb exposure was found to affect female reproductive function as demonstrated by both epidemiologic and toxicological studies. Some evidence is also available regarding associations between blood Pb levels and altered hormone levels in adults, but varied among studies. The differences may have been due to the different hormones examined and the different timing in the menstrual and life cycles of the women. Studies reported inconsistent findings for the association between Pb and fertility. Adjustment for potential confounders varies from study to study, with some potentially important confounders, such as BMI, not included in all studies. Also, many epidemiologic studies are limited by small samples sizes, and are generally comprised of women attending infertility clinics; which presents the possibility of selection bias and lack of generalizability. Toxicological studies found effects on female reproductive function after prenatal or early postnatal exposures. Further coherence for these findings in laboratory animal models is found in with findings in the ecological literature for the effects of Pb exposure on reduced fecundity in terrestrial and aquatic animal species (Sections 6.4.5.2, 6.3.4.2, 6.3.4.3, and 6.4.5.3). Although epidemiologic and toxicological studies provide information on different exposure periods, both types of studies support the conclusion that Pb affects at least some aspects of female reproductive function.

4.8.5 Summary and Causal Determination

Many epidemiologic and toxicological studies of the effects of Pb on reproductive and developmental outcomes have been conducted since the 2006 Pb AQCD. The evaluation of causal relationships with Pb exposure focuses on four areas: developmental effects, birth outcomes, reproductive function among males, and reproductive function among females. The sections that follow describe the evaluation of evidence for these outcomes with respect to causal relationships with Pb exposure using the framework described in Table II of the Preamble. The application of the key evidence to the causal framework is summarized in Table 4-48.

4.8.5.1 Effects on Development

The 2006 Pb AQCD (U.S. EPA, 2006b) reported Pb-associated effects on development in toxicological studies. Multiple epidemiologic studies of Pb and puberty have shown associations between concurrent blood Pb levels and delayed pubertal onset for girls and boys. Delayed puberty has been linked to decreased peak bone mass and increased risk of osteoporotic fractures (Gilsanz et al., 2011; Naves et al., 2005). In cross-sectional epidemiologic studies of girls (ages 6-18 years) with mean and/or median concurrent blood Pb levels from 1.2 to 9.5 µg/dL, consistent associations with delayed pubertal onset (measured by age at menarche, pubic hair development, and breast development) were observed. In boys (ages 8-15 years), fewer epidemiologic studies were conducted but associations between blood Pb levels and delayed puberty were observed, including associations among boys in a longitudinal study. These associations are consistently observed in populations with mean or median blood Pb levels of 3.0 to 9.5 µg/dL. Potential confounders considered in the epidemiologic studies of both boys and girls that performed regression analyses varied. Most studies controlled for age and BMI. Other variables, such as measures of diet, SES, and race/ethnicity, were included in some of the studies. Adjustment for nutritional factors was done less often and this could be an important confounder. A study using a cohort of girls from NHANES controlled for various dietary factors as well as other potential confounders and reported an association between increased concurrent blood Pb levels and delayed pubertal onset (Selevan et al., 2003). A limitation across most of the epidemiologic studies of blood Pb levels and delayed puberty is their cross-sectional design, which does not allow for an understanding of temporality. There is uncertainty with regard to the exposure frequency, timing, duration, and level that contributed to the associations observed in these studies.

Recent toxicological studies indicate that delayed pubertal onset may be one of the more sensitive developmental effects of Pb exposure with effects observed after gestational

exposures leading to blood Pb levels in the female pup of 1.3-13 µg/dL (Iavicoli et al., 2006a; Iavicoli et al., 2004). Toxicological studies have reported delayed male sexual maturity as measured with sex organ weight, among other outcomes, seeing significant decrements at blood Pb levels of 34 µg/dL (Sokol et al., 1985). Thus, data from the toxicological literature and from epidemiologic studies demonstrate that puberty onset in both males and females is delayed with Pb exposure.

Findings from epidemiologic studies of the effect of Pb on postnatal growth are inconsistent and findings from the toxicological literature of the effect of Pb exposure are mixed with recent growth findings showing adult onset male obesity after gestational and lactational Pb exposure (see Section 4.8.1.3). Toxicological studies demonstrated that other effects of Pb exposure during development include impairment of retinal development, effects on the lens of the eye, and alterations in the developing hematopoietic, hepatic systems and teeth.

The collective body of evidence integrated across epidemiologic and toxicological studies, based on the findings of delayed pubertal onset among males and females, is sufficient to conclude that there is a causal relationship between Pb exposure and developmental effects.

4.8.5.2 Effects on Birth Outcomes

Overall, results of pregnancy outcomes were similar to those of the 2006 Pb AQCD; (U.S. EPA, 2006b) inconsistent epidemiologic evidence of a relationship with Pb was available for preterm birth. The 2006 Pb AQCD reported potential associations between Pb and neural tube defects, but a recent epidemiologic study found no association. Some associations were observed between Pb and low birth weight when epidemiologic studies used measures of postpartum maternal bone Pb or air exposures. The associations were less consistent for maternal blood Pb measured during pregnancy or at delivery or umbilical cord and placenta Pb (maternal blood Pb or umbilical cord and placenta Pb were the biomarkers most commonly used in studies of low birth weight) but some associations between increased Pb biomarker levels and decreased low birth weight/fetal growth were observed. The effects of Pb exposure during gestation in animal toxicological studies included mixed findings with some studies showing reduction in litter size, implantation, and birth weight, and some showing no effect. Based on the mix of inconsistent results of studies on various birth outcomes but some associations observed in well-conducted epidemiologic studies of preterm birth and low birth weight/fetal growth, the evidence is suggestive of a causal relationship between Pb exposure and birth outcomes.

4.8.5.3 Effects on Male Reproductive Function

Toxicological evidence and supporting epidemiologic evidence indicate that a causal relationship exists between Pb exposure and effects on male reproductive function. Key evidence is provided by toxicological studies in rodents, non-human primates, and rabbits showing detrimental effects on semen quality, sperm and fecundity/fertility with supporting evidence in epidemiologic studies of associations between Pb exposure and detrimental effects on sperm. This is consistent with evidence reported in the 2006 Pb AQCD (U.S. EPA, 2006b).

Toxicological studies with relevant Pb exposure routes reported effects on rodent sperm quality and sperm production rate [(Sokol and Berman, 1991; Sokol et al., 1985), blood Pb level 34-37 µg/dL], sperm DNA damage [(Nava-Hernandez et al., 2009), blood Pb levels 19 and 22 µg/dL], and histological or ultrastructural damage to the male reproductive organs in studies from rodents [(El Shafai et al., 2011), blood Pb level 5.1 µg/dL] and non-human primates [(Cullen et al., 1993), blood Pb level 43 µg/dL]. These effects were found in animals exposed to Pb during peripuberty or adults for 1 week to 3 months. The toxicological studies reported an association between Pb exposure and decreases in reproductive organ weight and organ histological changes in the testes and germ cells. Subfecundity (decreased number of pups born/litter) was reported in unexposed females mated to Pb exposed males. Also, sperm from Pb-exposed rats (blood Pb level: 33 to 46 µg/dL) used for in vitro fertilization of eggs harvested from unexposed females yielded lower rates of fertilization. (Sokol et al., 1994). Supporting evidence was provided by decrements in sperm quality from rabbits administered Pb subcutaneously (blood Pb levels of 25 µg/dL) (Moorman et al., 1998).

Detrimental effects of Pb on sperm were observed in epidemiologic studies with concurrent blood Pb levels of 25 µg/dL and greater among men occupationally exposed (Hsu et al., 2009b; Kasperczyk et al., 2008; Naha and Manna, 2007; Naha and Chowdhury, 2006). The epidemiologic studies were limited due to these high exposure levels among the occupational cohorts and the lack of consideration for potential confounding factors, including occupational exposures other than Pb. Studies among men with lower Pb levels were limited to infertility clinic studies, which may produce a biased sample and findings that lack generalizability. However, a well-conducted epidemiologic study that enrolled men going to a clinic for either infertility issues or to make a semen donation and controlled for other metals as well as smoking reported a positive association with various detrimental effects in sperm (Telisman et al., 2007). The median concurrent blood Pb level in this study was 4.92 µg/dL (range: 1.13-14.91). A similar study (Meeker et al., 2008) also reported possible associations between concurrent blood

Pb and various semen parameters, but the results were extremely imprecise, making it difficult to draw conclusions.

Similar to the 2006 Pb AQCD (U.S. EPA, 2006b), recent epidemiologic and toxicological studies reported inconsistent results regarding hormone aberrations associated with Pb exposure. Due to the complexity of the reproductive system, uncertainty exists as to whether Pb exerts its toxic effects on the reproductive system by affecting the responsiveness of the hypothalamic-pituitary-gonad axis, by suppressing circulating hormone levels or by some other pathway. Mixed findings were also apparent among epidemiologic studies of fertility among men.

More recent toxicological studies suggest that oxidative stress is a major contributor to the effects of Pb exposure on the male reproductive system, providing mode of action support. The effects of ROS may involve interference with cellular defense systems leading to increased lipid peroxidation and free radical attack on lipids, proteins, and DNA. Several recent studies showed that Pb induced an increased generation of ROS within the male sex organs, and germ cell injury, as evidenced by aberrant germ cell structure and function. Co-administration of Pb with various antioxidant compounds either eliminated Pb-induced injury or greatly attenuated its effects. In addition, many studies that observed increased oxidative stress also observed increased apoptosis which is likely a critical underlying mechanism in Pb-induced germ cell DNA damage and dysfunction.

Based on the consistency and coherence of findings for the detrimental effects of Pb exposure on sperm and semen in the toxicological literature, the support from epidemiologic studies, and biological plausibility provided by mode of action evidence, the evidence is sufficient to conclude that there is a causal relationship between Pb exposures and male reproductive function.

4.8.5.4 Effects on Female Reproductive Function

Epidemiologic and toxicological studies of reproductive function among females investigated whether Pb biomarker levels were associated with hormone levels, fertility, estrus cycle changes, and morphology or histology of female reproductive organs including the placenta. Toxicological studies reported in the 2006 Pb AQCD reported associations between Pb exposure and female reproductive function, although little evidence was provided by epidemiologic studies (U.S. EPA, 2006b). Some epidemiologic studies have shown associations with concurrent blood Pb levels and altered hormone levels in adults, but results varied among studies, possibly due to the different hormones examined and the different timing in the menstrual and life cycles. There is some

evidence of a potential relationship between Pb exposure and female fertility, but findings are mixed. The majority of the epidemiologic studies are cross-sectional, and adjustment for potential confounders varies from study to study, with some potentially important confounders, such as BMI, not included in all studies. Also, most of the studies have small samples sizes and are generally of women attending infertility clinics. Toxicological study design often employs prenatal or early postnatal Pb exposures at relevant Pb levels, with Pb contributing to placental pathology and inflammation, decreased ovarian antioxidant capacity, altered ovarian steriodogenesis and aberrant gestational hormone levels. Although epidemiologic and toxicological studies provide information on different exposure periods, both types of studies, including some high-quality epidemiologic and toxicological studies, support the conclusion that Pb possibly affects at least some aspects of female reproductive function. Overall, the relationship observed with female reproductive outcomes, such as fertility, placental pathology, and hormone levels in some epidemiologic and toxicological studies, is sufficient to conclude that evidence is suggestive of a causal relationship between Pb exposure and female reproductive function.

Table 4-48 Summary of evidence supporting reproductive and developmental causal determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Exposure or Blood Pb Levels Associated with Effects[c]
Effects on Development – Causal			
Delayed Puberty Onset			
Consistent associations with relevant blood Pb levels in high-quality epidemiologic studies that help rule out chance, bias, and confounding with reasonable confidence	Consistent evidence in multiple large cross-sectional epidemiologic studies for females and males plus a longitudinal study for males. Most of these studies have large sample sizes and controlled for potential confounding by covariates such as age, BMI, and/or education/SES. There is uncertainty related to exposure patterns resulting in likely higher past Pb exposure	Tomoum et al. (2010), Hauser et al. (2008), Williams et al. (2010), Denham et al .(2005), Naicker et al. (2010), Wu et al. (2003b), Gollenberg et al. (2010) Sections 4.8.1.1 and 4.8.1.2	Concurrent blood Pb levels: 1.2-9.5 µg/dL
	A large study using females aged 8-18 years from the NHANES III study also controlled for various dietary factors and reported associations between blood Pb levels and delayed puberty onset	Selevan et al. (2003)	
Consistent toxicological evidence with relevant Pb exposures to rule out change, bias, and confounding with reasonable confidence.	Consistent toxicological evidence from multiple laboratories of delayed male and female puberty onset with Pb exposure via diet or oral gavage in rodents	Dumitrescu et al. (2008b), Iavicoli et al. (2006a), Pine et al. (2006) Sections 4.8.1.1 and 4.8.1.2	Blood Pb level after dietary exposure, or gavage, from gestation to estrus: 1.3 - 13 µg/dL
Evidence clearly describes mode of action.	Toxicological evidence describes HPG axis dysfunction and. Mode of action changes in IGF-1 contributing to Pb-induced delay in puberty onset.	Section 4.8.1.1	
Postnatal Growth			
Available toxicological evidence is mixed	There are mixed findings in the toxicological literature on Pb exposure and postnatal growth with some studies showing stunted growth in animals exposed to Pb and some showing no effects.	Section 4.8.1.3	22.8 µg/dL
	Recent toxicological evidence of effect of Pb on postnatal growth: obesity in adult male offspring after gestational + lactational Pb exposure		10 µg/dL 42 µg/dL

Table 4-48 (Continued): Summary of evidence supporting reproductive and developmental causal determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Exposure or Blood Pb Levels Associated with Effects[c]
Available epidemiologic evidence is inconsistent	Multiple studies, mostly cross-sectional, for children of varying ages have reported inconsistent results for the association between blood Pb levels and various measures of growth. There is uncertainty related to exposure patterns resulting in likely higher past Pb exposure.	Section 4.8.1.3	
Impaired Organ Systems			
Consistent toxicological evidence for effects on various organ systems but not always with relevant Pb exposures.	Relevant gestational and lactational Pb exposure of rats resulted in retinal ERG aberrations and increased retinal cell layer thickness. Effects also found on bone, teeth, and GI system.	Section 4.8.1.4	Blood Pb level after gestational-lactational exposure: 10-12 µg/dL
Effects on Birth Outcomes - Suggestive			
A few high-quality epidemiologic studies show associations with relevant blood Pb levels but findings are overall inconsistent.	Inconsistent findings for studies for birth defects, preterm birth, and low birth weight/fetal growth.	See Section 4.8.3 (and all subsections): 4.8.3.1, 4.8.3.2, 4.8.3.3 and 4.8.3.4	
	A few well-conducted epidemiologic studies of preterm birth and low birth weight/fetal growth using measures of maternal blood Pb at the time of pregnancy reported associations.	Jelliffe-Pawlowski et al. (2006), Vigeh et al.(2011), Zhu et al. (2010), Chen et al. (2006a), Gundacker et al. (2010)	Maternal pregnancy blood Pb levels: >10 µg/dL
Inconsistent toxicological evidence	The toxicological literature reported mixed findings with some studies showing smaller litter size (fewer pups born) or decreased birth weight with Pb exposure and some studies showing no effect.	See Sections 4.8.2.4 and 4.8.2.1	

Table 4-48 (Continued): Summary of evidence supporting reproductive and developmental causal determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Exposure or Blood Pb Levels Associated with Effects[c]
Effects on Male Reproductive Function – Causal			
Sperm/semen production, quality, and function			
High-quality and consistent toxicological evidence with relevant Pb exposures to rule out chance, bias, and confounding with reasonable confidence.	Decreased sperm counts, decreased sperm production rate, dose-dependent suppression of spermatogenesis in rodents with drinking water Pb exposure.	All results: Section 4.8.3.1 Sokol et al. (1985)	Blood Pb level after adult drinking water exposure for 30 days: 34 µg/dL
		Sokol and Berman (1991)	Blood Pb level after peripubertal or adult drinking water exposure for 30 days: 35 and 37 µg/dL.
	Ultrastructural and histological damage to non-human primates testis and seminiferous tubules.	Singh et al. (1993a) Foster et al. (1998)	Maximum blood Pb levels after daily oral Pb exposure (gelatin capsule) during infancy, post infancy, or over a lifetime (up to 10 yr): 32 to 36 µg/dL
	Histologic damage to rodent seminiferous tubules including spermatids and developing sperm.	El Shafai et al. (2011)	Blood Pb level after adult exposure (oral gavage) for 3 months: 5.31 µg/dL
	Ultrastructural abnormalities to rat spermatogenesis.	Murthy et al.(1995)	Blood Pb level after i.p. injection for 16 days: 7.4 µg/dL
	Direct effects on rodent sperm DNA after drinking water Pb exposure.	Nava-Hernández et al. (2009)	Blood Pb level after adult exposure for 13 weeks: 19 and 22 µg/dL
	Sperm from Pb exposed rats used for in vitro fertilization of eggs harvested from unexposed females yielded lower rates of fertilization.	Sokol et al.(1994)	Blood Pb level after adult exposure for 14-60 days: 33-46 µg/dL
	Semen and sperm quality in rabbits with subcutaneous Pb treatment; ultrastructural damage to spermatids with i.p. injection of Pb.	Moorman et al.(1998),	Blood Pb level after adult exposure for 15 weeks: 16-24 µg/dL
	Findings of detrimental effects of Pb exposure on sperm from multiple species (Deer, Asian earthworm, rainbow trout, marine worm, *H. elegans*, Fathead minnow)	See Ecological Effects; Sections 6.4.12.1 and 6.4.21.1)	

Table 4-48 (Continued): Summary of evidence supporting reproductive and developmental causal determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Exposure or Blood Pb Levels Associated with Effects[c]
Toxicological evidence is supported by consistent findings in epidemiologic studies that cannot rule out uncertainty	Consistent evidence from studies of occupational cohorts with high blood Pb levels. Results from occupational cohorts may have been confounded by other workplace exposures, which were not adjusted for in the epidemiologic studies. Potential confounding by smoking was considered in one study. There is uncertainty related to exposure patterns resulting in likely higher past Pb exposure.	Naha and Manna (2007), Naha and Chowdhury (2006), Hsu et al. (2009b), Kasperczyk et al. (2008)	Concurrent blood Pb levels: ≥ 25 µg/dL
	Results less consistent at lower blood Pb level. A well-conducted epidemiologic study at an infertility clinic reported associations between detrimental effects in sperm and blood Pb levels after controlling for smoking and other metal exposure. A similar study also reported some elevated effect estimates but the results were too imprecise to draw definitive conclusions. There is uncertainty related to exposure patterns resulting in likely higher past Pb exposure.	Telisman et al. (2007), Meeker et al. (2008)	Groups with concurrent blood Pb level: <15 µg/dL
Evidence describes mode of action.	Consistent evidence in reproductive organs of Pb-exposed male animals (of increased apoptosis of germ cells or spermatocytes), decreased antioxidant activity (SOD and CAT),and increased oxidative stress (MDA).	Sections 4.8.3.1 and 4.8.3.2	
Hormone levels			
Inconsistent findings in epidemiologic studies; few studies available	There are a small number of studies examining hormone levels and the results are inconsistent. There is uncertainty related to exposure patterns resulting in likely higher past Pb exposure.	Telisman et al. (2007), Naha and Manna (2007), Hsieh et al. (2009a), Meeker et al. (2010), Mendiola et al. (2011) Section 4.8.3.2	
Fertility			
Lack of large, well-conducted epidemiologic studies but overall inconsistent evidence	The few epidemiologic studies examining this outcome generally have small samples sizes and are drawn from men attending infertility clinics. There is uncertainty related to exposure patterns resulting in likely higher past Pb exposure.	Kiziler et al. (2007), Bloom et al. (2011b), Bloom et al. (2010), Gracia et al. (2005) Section 4.8.3.3	

Table 4-48 (Continued): Summary of evidence supporting reproductive and developmental causal determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Exposure or Blood Pb Levels Associated with Effects[c]
Limited toxicological evidence	Paternal Pb exposure resulted in less successful copulation, fewer implantations, and longer periods of time copulating for successful matings. Unexposed females with Pb-exposed male partners did not have fewer pregnancies, but did produce smaller litters.	Anjum et al. (2010) Sainatha et al. (2011) Pace et al. (2005) Section 4.8.3.3	45-day exposure of adult male rats to 500 or 1,500 ppm Pb acetate exposure in drinking water, followed by behavioral mating studies with unexposed females.
Effects on Female Reproductive Function - Suggestive			
A few high-quality epidemiologic studies of Pb levels and hormones demonstrate associations but are inconsistent overall	Evidence in some high-quality cross-sectional epidemiologic studies demonstrates associations with hormone levels but results are mixed and vary by hormone examined and timing in a woman's menstrual and life cycles. In addition, the potential confounders vary from study to study, with some potentially important confounders, such as BMI, not included in all studies. There is uncertainty related to exposure patterns resulting in likely higher past Pb exposure.	Jackson et al. (2011), Pollack et al. (2011), Chang et al. (2006), Krieg (2007) Section 4.8.4.1	Concurrent mean blood Pb levels: <5 µg/dL
Lack of large, well-conducted epidemiologic studies examining associations between Pb levels and fertility, but overall inconsistent evidence	Epidemiologic studies of this association are limited by the small sample sizes included in those studies. In addition, most of the study populations were drawn from women undergoing IVF and/or attending infertility clinics. There is uncertainty related to exposure patterns resulting in likely higher past Pb exposure.	Section 4.8.4.2	
Toxicological studies of Pb and effects on female reproduction demonstrate effects in some studies.	Evidence in the toxicological literature of Pb contributing to placental pathology and inflammation, decreased ovarian antioxidant capacity, altered ovarian steriodogenesis and aberrant gestational hormone levels.	Section 4.8.4.1 and 4.8.4.3	

[a]Described in detail in Table II of the Preamble.

[b]Describes the key evidence and references contributing most heavily to causal determination and where applicable to uncertainties or inconsistencies. References to earlier sections indicate where full body of evidence is described.

[c]Describes the blood Pb level in humans with which evidence is substantiated or the blood Pb levels or Pb exposure concentrations in animals relevant to this ISA.

4.9 Effects on Other Organ Systems

The 2006 Pb AQCD described limited evidence for the effects of Pb exposure on various organ systems including the liver, GI tract, endocrine system, bone and teeth, eyes, and respiratory tract. These lines of evidence largely are supported by recent toxicological and epidemiologic studies, although the collective evidence remains relatively limited in terms of the quantity and design of studies and/or the populations examined.

4.9.1 Effects on the Hepatic System

Hepatotoxic effects of Pb exposure (indicated in various animal models and/or human populations) include alterations in hepatic metabolism, hepatic cell proliferation, changes in cholesterol metabolism, as well as oxidative stress-related injury.

4.9.1.1 Summary of Key Findings of the Effects on the Hepatic System from the 2006 Pb AQCD

The 2006 Pb AQCD (U.S. EPA, 2006b) stated that the experimental animal database indicated hepatotoxic effects, including liver hyperplasia, at very high dose Pb exposures. Other effects noted in the liver following exposure to Pb included altered cholesterol synthesis, DNA synthesis, and glucose-6-phosphotase dehydrogenase (G6DP) activity. The 2006 Pb AQCD reported that cytochrome P450 (CYP) levels decreased following single doses of Pb nitrate (20-100 μmol/kg). Induced and constitutive expression of microsomal CYP1A1 and CYP1A2 was inhibited by Pb exposure. Inhibition of these (Phase I) xenobiotic metabolizing enzymes was accompanied by an increase in Phase II enzymes following exposure to Pb nitrate and other Pb compounds, suggesting that Pb is capable of inducing a biochemical phenotype similar to hepatic nodules. Studies investigating Pb-induced hepatic hyperplasia suggested alterations in the gluconeogenic mechanism, DNA hypomethylation, changes in proto-oncogene expression, as well as altered cholesterol synthesis. Cholesterol metabolism changes following exposure to Pb were reportedly mediated by induction of several enzymes related to cholesterol metabolism as well as a decrease in the cholesterol catabolizing enzyme, 7 α-hydroxylase. Tumor necrosis factor alpha (TNF-α) was reported to be one of the major mitogenic signals that mediated Pb nitrate-induced hepatic hyperplasia, based on findings showing that inhibitors blocking TNF-α activity also blocked Pb-induced hyperplasia. Other Pb-related effects presented in the 2006 Pb AQCD included liver cell apoptosis and Pb-induced oxidative stress in in vitro cell cultures. The 2006 Pb AQCD

further suggested that alterations in liver heme metabolism may involve changes in 5-aminolevulinic acid dehydrogenase (ALAD) activity, porphyrin metabolism, transferrin gene expression and changes in Fe metabolism.

With regard to human studies, the 2006 Pb AQCD stated that increases in serum liver enzymes suggest that Pb exposure (> 50 µg/dL) results in nonspecific liver injury in occupationally-exposed adults. However, studies did not adjust for potential confounding factors, including other occupational exposures or establish explicit associations between Pb exposure and hepatic injury (i.e., observation of histopathological effects). In addition, similar effects were noted in animal studies, and decreased CYP activity was associated with higher blood Pb levels in a few studies of children or adults (drawn from the general population). The 2006 Pb AQCD reported that hepatic effects in humans were associated only with high blood Pb levels, i.e., >30 µg/dL.

4.9.1.2 Recent Epidemiologic Studies

A few recent epidemiologic studies examined antioxidant status and oxidative stress effects, as measured by liver biochemical parameters, associated with occupational exposure to Pb. However, all of these occupationally-exposed cohorts represented populations highly exposed to Pb, resulting in mean or median blood Pb levels ranging from 29 to 53 µg/dL. Although the hepatic effects observed within these occupational cohorts do not reflect the general population as a whole, they are useful in demonstrating consistent effects on a number of liver outcomes, including altered liver function (i.e., changes in the level of liver function enzymes), oxidative stress, and antioxidant status (Can et al., 2008; Khan et al., 2008; Patil et al., 2007). However, these studies were cross-sectional in design with concurrent blood Pb measurement. Thus, there is uncertainty regarding the directionality of effects and in the magnitude, timing, frequency, and duration of Pb exposure that contributed to the observed associations. Further, these cross-sectional analyses did not consider potential confounding by factors such as age, diet, BMI, smoking, or other occupational exposures.

Patil et al. (2007) reported that concurrent (SD) blood Pb levels (mean [SD]: 22.32 [8.87] µg/dL) were significantly higher ($p < 0.001$, t-test) in 30 spray painters from Kolhapur City in western Maharashtra, India (exposed to Pb for >6 hours/day for 2 to 20 years) than blood Pb levels (mean [SD]: 12.52 [4.08] µg/dL) in the 35 concurrent controls who had no history of Pb exposure and lived in rural areas. Levels of liver function enzymes, including the two serum transaminase enzymes SGOT (also known as AST; serum glutamic oxaloacetic transaminase/aspartate aminotransferase) and SGPT (also known as ALT; serum glutamic pyruvic transaminase/alanine aminotransferase),

were increased in spray painters compared to controls, whereas total serum protein levels were decreased compared to controls ($p < 0.01$, t-test). In another occupational study, Conterato et al. (2013) investigated liver function parameters in automotive industry painters and in battery manufacturing workers, who were exposed to Pb in Brazil. Mean (SD) concurrent blood Pb levels were 5.4 (0.4) µg/dL in the 50 Pb-exposed painters and 1.5 (0.1) µg/dL in the 36 unexposed controls. The mean (SD) duration of exposure to Pb in automotive industry painters was 133.9 (14.5) months (~11 years). In the Pb-exposed paint workers, the levels of AST (but not γ-glutamyltransferase), were increased nearly 2-fold compared to AST levels in controls ($p < 0.05$). The activity of AST was positively correlated with blood Pb levels (Spearman $R = 0.26$, $p < 0.05$). The authors suggested that confounding exposures to other toxic constituents of the paints regularly used by painters, and not Pb, may be the etiological cause of decrements in AST function as these effects were not also seen in battery workers with much higher blood Pb levels (49.8 µg/dL) (Conterato et al., 2013). Co-exposure to other environmental contaminants may also explain the effects that were previously reported in occupationally-exposed spray-painters in Patil et al. (2007).

4.9.1.3 Recent Toxicological Studies

Hepatic Metabolism

As stated in the 2006 Pb AQCD (U.S. EPA, 2006b), acute (e.g., single dose, 5 to 10 mmol Pb/kg body wt) treatment of rodents with Pb nitrate and other Pb compounds was found to result in a decrease in Phase I enzymes and a simultaneous increase in Phase II enzymes. The conclusions presented in the 2006 Pb AQCD were also reviewed by Mudipalli (2007).

Recent studies found changes in biochemical parameters, suggestive of liver damage, in animals exposed to Pb; however, the relevance to humans is often uncertain because of the high blood Pb levels examined in animal studies and/or the gavage or injection routes of Pb administration used in some of the animal studies. Undernourished male Wistar rats (fed low-protein diet without mineral supplements) exposed to 500 ppm Pb acetate in drinking water over a 10 month period had decreases in serum protein and albumin levels as well as increases in AST, ALT, serum alkaline phosphatase (ALP), and gamma glutamyl transpeptidase (GGT) levels (Herman et al., 2009). In the Pb-treated animals, the blood Pb levels steadily increased throughout the initial portion of the study period, reaching a maximum of approximately 30 µg/dL after 2 months. After this time, blood Pb levels rapidly increased to approximately 110 µg/dL by six months time, and remained at this level until the termination of exposure at 10 months. Similar biochemical changes

were not observed in animals treated with Pb acetate maintained on protein-adequate, mineral rich diet.

Similarly, mice gavaged with Pb nitrate (50 mg/kg for 40 days) also demonstrated increased activities of AST, ALT, ALP, and acid phosphatase (ACP) compared to controls (Sharma et al., 2010a). Upadhyay et al. (2009) reported that treatment of Sprague-Dawley rats with Pb acetate (35 mg/kg via i.p. injection for 3 days, resulting blood Pb level not reported) significantly increased the activities of ALT, AST, serum ALP, and acid phosphatase over those in controls but decreased liver ALP activity. Concomitant treatment with Zn and varying levels of vitamin C were observed to ameliorate these toxic effects of Pb. The serum activities of glutamic pyruvic transaminase (GPT) and lactate dehydrogenase (LDH) were similarly significantly increased over those in controls in mice subcutaneously (s.c.) injected with Pb acetate (50 mg/kg daily for 15 days, resulting blood Pb levels not reported) (Wang et al., 2010h). Swarup et al. (2007) investigated serum biochemical changes in cows living in Pb-contaminated environments. Serum levels of ALT, AST, ALP, total protein, albumin, globulin, and A/G ratio were significantly altered in cows living near Pb-Zn smelters (mean [SD] blood Pb level: 86 [6] µg/dL) compared to control cows (mean [SD] blood Pb level: 7 [1] µg/dL). Significant positive correlations were found between blood Pb level and ALT and AST levels, whereas a negative correlation was observed between blood Pb level and total lipids, protein, and albumin.

Pillai et al. (2009) investigated hepatic Phase I and II enzymes in male and female rats born to dams that were treated with Pb acetate (50 µg/kg, via s.c. injection daily throughout gestation and continuing until PND21). Thus, the offspring of treated dams were exposed to Pb via placental and lactational transfer. The female and male pups were then allowed to reach sexual maturity (PND55-PND56), in order to assess continuing exposure to bioaccumulated Pb. The activities of hepatic Phase I enzymes NADPH- and NADH-cytochrome *c* reductase were significantly reduced in Pb-exposed male and female rats on PND56 (resulting blood Pb levels not reported), compared to controls. In rats treated with Pb and Cd (25 µg/kg), the effect on Phase I enzymes was increased. Pb treatment additionally decreased the activities of Phase II enzymes uridine diphosphate-glucoronyl transferase and GST in males and females, but no effect was observed on GGT or 17β-hydroxysteroid oxidoreductase. Additionally, no effect was observed on serum glutamate pyruvate dehydrogenase or ALP activities in the Pb-treated male or female rats. Histological observations demonstrated fatty degeneration of the liver, vacuolization, and pycnotic nuclei, indicating general hepatotoxicity following Pb treatment in both male and female rats.

In a similar study, Teijon et al. (2006) exposed Wistar rats to Pb acetate (200 or 400 ppm drinking water) throughout gestation, lactation, and continuing through 1 and 3 months postweaning. Compared to the controls, the concentrations of Pb in the liver of the male pups were elevated in the 200- and 400-ppm groups at both 1 and 3 months postweaning (only male pups were followed after birth). Liver concentrations of Pb were greater in the 200 ppm animals compared to the 400 ppm animals at one month postweaning (mean [SE]: 1.19 [0.30] µg Pb/g tissue versus 0.76 [0.06] µg Pb/g tissue, respectively), but were similar between the 2 dosing regimens (200 ppm versus 400 ppm) by 3 months postweaning (mean [SE]: 0.54 [0.06] versus 0.55 [0.07] µg Pb/g tissue, respectively). Serum ALP activity was significantly increased over controls only at 2 weeks postweaning, whereas ALT activity was significantly decreased from controls only at 2 months and at 3 months postweaning. Cheng et al. (2006) studied the mechanism of Pb effects on bacterial lipopolysaccharide (LPS)-induced TNF-α expression. A/J mice were injected with Pb acetate (100 µmol/kg via i.p.), with or without LPS (5 mg/kg). Pb alone did not affect AST or ALT activity or the level of TNF-α in the serum of the mice. In comparison, treatment of mice with low doses of Pb and LPS together caused a statistically significant increase in TNF-α induction as well as enhanced liver injury, suggesting that Pb potentiated LPS-induced inflammation. In a complementary in vitro experiment, co-exposure of Pb and LPS stimulated the phosphorylation of p42/44 mitogen-activated protein kinase (MAPK) and increased TNF-α expression in mouse whole blood cells, peritoneal macrophages, and RAW264.7 cells (a macrophage cell line). These results indicated that Pb increased LPS-induced TNF-α levels via the protein kinase C (PKC)/MAPK pathway in monocytes/macrophages rather than hepatocytes. Similarly, Pb chloride potentiated bovine serum albumin (BSA)-induced inflammation in the livers of mice subcutaneously injected with Pb (Sá et al., 2012).

Lipid Metabolism

Several recent toxicological studies indicated Pb-induced impaired lipid metabolism, as evidenced by increases in liver cholesterol. There was some evidence in animals exposed to Pb in diet, albeit at relatively high exposure concentrations or measured blood Pb levels. Ademuyiwa et al. (2009) reported that male albino Sprague Dawley rats exposed to 200, 300 and 400 ppm Pb acetate in drinking water had mean (SD) blood Pb levels of 40.63 (9.21), 61.44 (4.63), and 39.00 (7.90) µg/dL, respectively. Animals exposed to 200 ppm Pb had mean (SD) liver Pb concentrations of 10.04 (1.14) µg/g, compared to 3.24 (1.19) µg/g and 2.41 (0.31) µg/g in animals exposed to 300 or 400 ppm Pb, respectively. The animals exposed to Pb exhibited increased hepatic cholesterogenesis at all doses tested compared to controls. Additionally, a decrease in triglyceride levels was observed at 300 and 400 ppm Pb; a decrease in phospholipid levels was observed at

400 ppm Pb. The authors also reported positive correlations between tissue cholesterol and phospholipids, and Pb accumulation in liver across all doses. In contrast, the association between tissue triglyceride levels and Pb accumulation was negative. In related studies, Khotimchenko and Kolenchenko (2007) reported that adult male albino rats treated with Pb acetate (100 mg/kg for 14 days, resulting blood Pb levels not reported) exhibited disorders in lipid metabolism that were accompanied by increased levels of total cholesterol and triglycerides in the liver tissue. Sharma et al. (2010a) reported increased liver cholesterol in mice gavaged with Pb nitrate (50 mg/kg for 40 days). Pillai et al. (2009) observed decreases in total liver cholesterol in PND56 male and female rats that had been treated with Pb acetate (via s.c. injection, 50 µg/kg, continuously throughout gestation and lactation). These results suggest that Pb induction of cholesterogenesis and phospholipidosis in the liver may cause subtle effects at the cellular level that may lead to hepatotoxicity.

Kojima and Degawa (2006) examined sex-related differences in Pb-induced gene expression of a rate limiting hepatic cholesterol biosynthesis enzyme, 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) and its transcription factor, sterol regulatory element binding protein-2 (SREBP-2). Male and female Sprague Dawley rats were injected with Pb nitrate (100 µmol/kg body weight, intravenously [i.v.], resulting blood Pb levels not reported). SREBP-2 expression was significantly increased in males and females with this increase occurring earlier in male rats (6-12 hours, compared to 24-36 hours in females). In contrast, expression of HMGR was significantly increased in both Pb-exposed males and females at earlier time frames and greater range of onset (3-48 hours in males; 12-48 hours in females) compared to that of SREBP-2. Significant increases in total liver cholesterol were also observed in Pb-exposed males and females at 3-48 and 24-48 hours, respectively. These results suggest that the SREBP-2 and HMGR gene expression increases and increase in total cholesterol levels in the liver, in response to Pb exposure, all occur earlier in males compared to females; and also suggest that the increase in HMGR gene expression and increase in total cholesterol levels in the liver occur before an increase in the SREBP-2 gene expression in both sexes.

Hepatic Oxidative Stress

A number of studies demonstrated increased hepatic oxidative stress as a result of exposure to various Pb compounds, measured by increases in reactive oxygen species (ROS) or decreases in antioxidant levels or enzyme activity. ROS can potentially result in damage to hepatic function and structure. Several of these observations were made in animals exposed to Pb in drinking water that produced blood Pb levels relevant to this ISA. In a study examining the effects of Pb exposure to fetuses, Massó et al. (2007) exposed pregnant Wistar rats to 300 ppm Pb in drinking water from GD1 to parturition

(PND0), or to weaning (PND21). Blood Pb levels were higher at parturition (mean [SD]: 31.5 [0.80] µg/dL) than at weaning (mean [SD]: 22.8 [0.50] µg/dL). Pups exhibited liver damage that was accompanied by an increased production of thiobarbituric acid-reactive species (TBARS, an indicator of lipid peroxidation) and increased CAT activity compared to controls. In addition, increased ALP and acid phosphatase activity was observed. Uzbekov et al. (2007) showed differential effects by duration of maternal exposure before mating. Female Wistar rats were exposed to 0.3 and 3.0 ppm Pb nitrate in drinking water, for 1 month or 5 months prior to pregnancy, and also continuing during pregnancy; then the livers from both groups of GD20 fetuses were examined for hepatic SOD activity. The pregnant control rats had a mean (SD) blood Pb level of 16.1 (0.63) µg/dL, whereas the dams exposed to 0.3 and 3.0 ppm Pb had mean blood Pb levels of 20.4 µg/dL and 24.4 µg/dL, respectively. In the GD20 fetuses from dams exposed for 1 month prior to pregnancy, a concentration-dependent increase in liver SOD activity was observed, whereas SOD activity was decreased in the GD20 fetuses from dams exposed for 5 months prior to pregnancy. The increase in SOD activity in the livers of fetuses from dams exposed to 0.3 or 3.0 ppm Pb nitrate for one month suggests an initial activation of SOD in response to increased free radical production, while the decrease in SOD production in fetal livers from dams exposed to the same concentrations for 5 months suggests that longer durations of Pb exposure impairs the antioxidant defense mechanism.

Increased hepatic oxidative stress was also found in animals with postnatal Pb exposure in drinking water. Jurczuk et al. (2007) reported that adult male Wistar rats (PND56) treated for 12 weeks with 500 ppm Pb in drinking water (resulting blood Pb level not reported) exhibited decreases in liver vitamin E and GSH levels along with an increase in lipid peroxidation. The correlation between vitamin E and lipid peroxidation suggested that vitamin E is involved in the mechanism of peroxidative action of Pb in the liver. In a study examining the role of low molecular weight thiols on peroxidative mechanisms, Jurczuk et al. (2006) found that adult male Wistar rats (PND56) treated for 12 weeks with 500 ppm Pb acetate in drinking water exhibited a decrease in blood ALAD as well as decreases in GSH and nonprotein sulfhydryl levels in the liver. Metallothionein levels were also reported to be higher in the liver following exposure to Pb. Yu et al. (2008) reported concentration-dependent increases in lipid peroxide levels and decreases in GSH levels and CAT, SOD and GPx activities in livers from castrated male pigs that received a diet mixed with 0, 5, 10, or 20 mg/kg Pb nitrate exposure, during ages 62-107 days. The level of hepatic CuZnSOD mRNA was also reduced in Pb-treated animals. The study authors suggested that this decrease in SOD mRNA expression and activity of antioxidant enzymes may lead to a reduction in free radical scavenging capability and increased lipid peroxidation.

Studies administering Pb by bolus doses had similar findings. Adegbesan and Adenuga (2007) reported that lipid peroxidation was increased and SOD activity was decreased in protein undernourished male Wistar rats compared to well-fed control rats, and that these effects were further exacerbated in protein undernourished rats receiving a single i.v. injection of Pb nitrate (100 µmol/kg, resulting blood Pb levels not reported). Protein undernourishment also decreased GSH levels and CAT activity compared to normal diet; however, co-treatment with Pb mitigated the severity of these effects. GSH levels and CAT activity were still lower in undernourished rats with Pb exposure compared to well-fed rats, but greater than undernourished rats with no Pb exposure. The results indicated Pb treatment exacerbated the effects of malnutrition on liver lipid peroxidation and altered the involvement of free radicals. Adult male Charles-Foster rats treated for 15 days with Pb acetate (0.025 mg/kg per day [via i.p. injection], resulting blood Pb levels not reported) also exhibited statistically significant increases in lipid peroxidation levels and decreases in SOD, CAT, and G6PD levels in liver mitochondrial and postmitochondrial fractions (Pandya et al., 2010). Statistically nonsignificant decreases were observed in GSH levels, and in GPx and GR activities in these Pb-treated rats. In mice gavaged with Pb nitrate (50 mg/kg for 40 days), lipid peroxidation was increased, and SOD, CAT, and GSH were decreased compared to controls (Sharma et al., 2010a). Additionally, Pb nitrate treatment resulted in histopathological changes in the structure of the liver: hepatocytes were damaged and were marked by cytoplasmic vacuolization and pycnotic nuclei.

Khotimchenko and Kolinchenko (2007) also reported an increase in lipid peroxidation and development of hepatitis in adult male albino rat liver parenchyma following treatment with Pb acetate (100 mg/kg per day, via gavage, for 14 days [resulting blood Pb levels not reported]). The increased lipid peroxidation was measured by increases in malondialdehyde (MDA) levels along with decreases in GSH and thiol groups; indicating injury in the liver antioxidant system. In another study, levels of hepatic lipid peroxidation were observed to be significantly increased in rats injected (i.p.) with Pb acetate (35 mg/kg per day for 3 days, resulting blood Pb levels not reported), whereas hepatic GSH was significantly decreased (Upadhyay et al., 2009). A study examining male and female rat pups that were continuously exposed to Pb during gestation and lactation (pregnant dams were injected [s.c.] with Pb acetate, 50 µg/kg per day [GD0 to PND21], resulting blood Pb levels not reported), did not find effects on GSH or MDA levels at PND56 (Pillai et al., 2009). In vitro exposure of cells from a hepatic human embryonic epithelial cell line (WRL-68) to 5 µM Pb acetate for 30 days resulted in increased production of ROS throughout the incubation period (Hernández-Franco et al., 2011). Concurrent with this increase in ROS generation, the activities of SOD and the levels of membrane lipid peroxidative damage increased throughout the first 24 days of in vitro exposure but returned to normal levels by exposure day 30.

Hepatic Apoptosis

Fan et al. (2009b) reported that a single i.v. injection of Pb nitrate (200 µmol/kg in 0.5 mL) in adult male Sprague-Dawley rats resulted in an increase in the percentage of apoptotic hepatocytes (mean: 2.5% [SD: 1.4] of total hepatocytes) compared with controls (mean: 0.31% [SD: 0.31]). Expression of ferritin light-chain (FTL) protein also increased (mean [SD]: 3.5 [1.0]-fold increase) over that in controls. Immunohistochemical analysis revealed that hepatocytes around the hepatic lobules' central veins were heavily stained by anti-FTL antibodies, as were nonparenchymal cells identified as Kupffer macrophage cells. The authors hypothesized that the expression of FTL in Kupffer cells may have resulted from the phagocytic engulfment of the apoptotic cells by the activated Kupffer cells. Treatment of rats with clofibrate, a lipid lowering agent, also increased FTL protein in hepatocytes around the central veins, but did not increase FTL expression in Kupffer cells; and induced hepatocellular proliferation, but not apoptosis.

4.9.1.4 Summary of Effects on the Hepatic System

While explicit associations between hepatic injury (i.e., histopathological effects) and Pb exposure have not been established, evidence from epidemiologic and toxicological studies have indicated that exposure to Pb can result in altered liver function and hepatic oxidative stress. A few studies have reported associations between higher blood Pb levels and decreased CYP enzymes (Phase I xenobiotic metabolism) in children and nonoccupationally-exposed adults. However, most occupational evidence indicates decreases in serum protein and albumin levels and increased AST, ALT, ALP, and GGT activities (indicators of decreased liver function), increased oxidative stress, and decreased antioxidant status in Pb-exposed workers with blood Pb levels >29 µg/dL (Conterato et al., 2013; Can et al., 2008; Khan et al., 2008; Patil et al., 2007). The implications of the occupational epidemiologic evidence are limited because of the cross-sectional design of the studies, the high blood Pb levels examined, and the lack of consideration for potential confounding by factors such as age, diet, BMI, smoking, or other occupational exposures.

Similar changes in liver function enzymes have been found in mature animals exposed to high levels of Pb during adulthood (Sharma et al., 2010a; Wang et al., 2010h; Herman et al., 2009; Cheng et al., 2006), and animals exposed during gestation and lactation (Pillai et al., 2009; Teijón et al., 2006). Pb exposure has been shown to impair lipid metabolism in animals, as evidenced by increased hepatic cholesterogenesis, and altered triglyceride and phospholipid levels (Ademuyiwa et al., 2009; Khotimchenko and Kolenchenko,

2007). Multiple studies in humans and animals have observed Pb-associated increases in hepatic oxidative stress, generally indicated by an increase in lipid peroxidation along with a decrease in GSH levels and CAT, SOD, and GPx activities (Pandya et al., 2010; Sharma et al., 2010a; Khan et al., 2008; Yu et al., 2008; Adegbesan and Adenuga, 2007; Jurczuk et al., 2007; Khotimchenko and Kolenchenko, 2007; Jurczuk et al., 2006). Indices of increased oxidative stress were also observed in the livers of rat fetuses exposed to Pb throughout gestation (Massó et al., 2007). The relevance of the toxicological evidence is uncertain, as many studies administered Pb as bolus doses. Additionally, few toxicological studies reported resulting blood Pb levels. Of those studies that did report blood Pb levels, most were so high (>30 µg/dL) as to limit their relevance to humans. Because of the insufficient quality of studies, the evidence is inadequate to determine if there is a causal relationship between Pb exposure and hepatic effects.

4.9.2 Effects on the Gastrointestinal System

Gastrointestinal effects examined in relation to Pb exposure include abdominal pain, constipation, and internal paralysis in humans and degeneration of the intestinal epithelial mucosa and a decrease in duodenal motility in animals.

4.9.2.1 Summary of Key Findings on the Effects on the Gastrointestinal System from the 2006 Pb AQCD

The 2006 Pb AQCD (U.S. EPA, 2006b) stated that a number of factors influence the gastrointestinal absorption of Pb; including the chemical and physical form of Pb, the developmental age at Pb intake, as well as various nutritional factors.

Rats exposed to Pb acetate in drinking water (0.1%) had degeneration of the intestinal epithelial mucosa, potentially leading to malabsorption of nutrients. Uptake of Pb via oral exposure was shown to be dependent on source of Pb. Pb administered in bovine or rat milk, or infant milk formula is associated with casein micelles, and was observed to accumulate in ileal tissues, whereas Pb administered in drinking water as a soluble salt was observed to accumulate in duodenal tissues. This difference in Pb uptake suggests that Pb exposure via drinking water may be more toxic than Pb exposure via milk. In a different study reviewed in the 2006 Pb AQCD, decreases in duodenal motility and the amplitude of contractility in the intestinal tract were observed in rats following Pb acetate exposure (gavage, 44 mg/kg per day for 4 weeks) . Nutritional studies using chicks examining different dietary levels of Pb, Ca^{2+}, and vitamin D in rats indicated

competition in absorption between Pb and Ca^{2+}. Dietary supplementation with vitamin D led to an increase in intestinal absorption of Pb and Ca^{2+}. In instances where severe Ca^{2+} deficiency was noted, ingestion of Pb caused a clear decrease in 1,25-dihydroxy vitamin D (1,25-$(OH)_2$D3) levels. Overall, the 2006 Pb AQCD stated that studies in rat intestine have shown that the largest amount of Pb absorption occurs in the duodenum with the mechanisms of absorption involving active transport and diffusion via the intestinal epithelial cells. Absorption has been reported to occur, through both saturable and nonsaturable pathways, based on results from various animal studies.

The 2006 Pb AQCD also reported on human evidence that symptoms associated with gastrointestinal colic (abdominal pain, constipation, intestinal paralysis) were more prevalent in occupationally-exposed adults with blood Pb levels ≥ 50 µg/dL.

4.9.2.2 Recent Epidemiologic Studies

Consistent with previous occupational findings, Kuruvilla et al. (2006) reported gastrointestinal effects in men, including stomach pain and gastritis along with other non-gastrointestinal effects in 53 Pb-exposed paint workers (mean [SD] blood Pb: 8.04 [5.04] µg/dL) in India compared with 50 controls (mean [SD] blood Pb level: 5.76 [4.45] µg/dL) matched by sex, age, education, income, smoking, and alcohol consumption. Fifty-two male battery workers were also examined. Prevalence of gastrointestinal symptoms in painters did not differ from that in battery workers with higher blood Pb levels (mean [SD] blood Pb level of 42.40 ([25.53] µg/dL). Despite the consistent evidence with earlier occupational studies, the implications of gastrointestinal findings in Pb-exposed workers are limited by the cross-sectional study designs, high blood Pb levels associated with effects (mostly ≥ 50 µg/dL), and limited consideration for potential confounding by factors such as age, smoking, alcohol use, nutrition, or other occupational exposures.

4.9.2.3 Recent Toxicological Studies

A few recent studies pertaining to gastrointestinal effects of Pb exposure were identified that provide evidence for additional mechanisms underlying gastrointestinal damage and impaired function. Santos et al. (2006) examined the impact of Pb exposure on relaxations to nonadrenergic noncholinergic (NANC) nerve stimulation in rat gastric fundus. Male Wistar rats treated with 80 ppm Pb acetate via drinking water for 15, 30, and 120 days (resulting blood Pb levels not reported) exhibited a significant difference in NANC relaxations in the gastric fundus following electrical field stimulus. While

frequency-dependent (0.5 - 8 Hz) relaxations were observed in all groups, including the control group, the relaxations were significantly inhibited in rats treated with the 80 ppm Pb acetate for all three durations (15, 30, and 120 days). When gastric fundus strips from rats were incubated with L-nitroarginine, a nitric oxide (NO) synthase inhibiter, no additional inhibition in relaxations was observed. In contrast, incubation with sodium nitroprusside and 8-Br-GMPc (a cyclic guanosine monophosphate [cGMP] analog) resulted in a concentration-dependent relaxation in strips from the control group and from the group that had been exposed to the 80 ppm Pb acetate for 120 days. The results suggested that long-term exposure to Pb causes inhibition in NANC relaxation, probably due to the modulated release of NO from the NANC nerves or due to interaction with the intracellular transducer mechanism in the rat gastric fundus.

In a study examining Pb-induced oxidative stress in the gastric mucosa, Olaleye et al. (2007) treated young adult (80–90 grams) male albino Wistar rats with 100 or 5,000 ppm Pb acetate in drinking water for 15 weeks (resulting blood Pb levels not reported). After the 15 week Pb acetate treatment, gastric ulcers were induced by a 4-hour exposure to acidified ethanol. The 15-week pre-exposure to Pb acetate led to a significant increase in gastric mucosal damage induced by the acidified ethanol, compared to the damage in the control group. Gastric ulcers were not induced in all of the animals. After the 15 week exposure to Pb acetate (but before any acidified ethanol treatment), stomach gastric acid secretion and biochemical analyses were performed in some of the rats. While the basal gastric acid secretory rate was not altered, stomach response to histamine was significantly higher in animals treated with the Pb acetate compared to that in the controls. Additionally, there was a significant increase in gastric lipid peroxidation at both the 100 and 5,000 ppm dosage levels. In contrast, CAT, and SOD activities and nitrite levels were significantly decreased in the gastric mucosa. These results indicated that Pb-induced gastric damage may be mediated via increases in oxidative stress.

4.9.2.4 Summary of Gastrointestinal Effects

Relatively few human studies have been conducted on the gastrointestinal toxicity of Pb. The evidence points to more prevalent symptoms, such as stomach pain, gastritis, constipation, and intestinal paralysis, in Pb-exposed workers (Kuruvilla et al., 2006). However, the implications of gastrointestinal findings in Pb-exposed workers are limited by the cross-sectional study designs, high blood Pb levels associated with effects (mostly $\geq$ 40 µg/dL), and limited consideration of potential confounding by factors such as age, smoking, alcohol use, nutrition, or other occupational exposures. Toxicological evidence indicates that Pb is absorbed primarily in the duodenum by active transport and diffusion, although variability is observed by Pb compound, age of intake, and nutritional factors.

There is some coherence between the evidence in Pb-exposed workers and observations in animals that Pb induces damage to the intestinal mucosal epithelium, decreases duodenum contractility and motility, reduces absorption of Ca^{2+}, inhibits NANC relaxations in the gastric fundus, and induces oxidative stress (lipid peroxidation, decreased SOD and CAT) in the gastric mucosa (Olaleye et al., 2007; Santos et al., 2006). The observation of oxidative stress was accompanied by gastric mucosal damage. Because of the insufficient quantity and quality of studies, the evidence is inadequate to determine if there is a causal relationship between Pb exposure and gastrointestinal effects.

4.9.3 Effects on the Endocrine System

A summary of key findings pertaining to reproductive hormones in males and females is presented in the section on Reproductive and Developmental Effects (Sections 4.8.3 and 4.8.4). Collective epidemiologic and toxicological evidence is inconsistent in demonstrating the effects of Pb exposure on male and female sex hormone levels. Other endocrine processes that are most commonly found to be impacted by Pb exposure include changes in thyroid hormones, including thyroid stimulating hormone (TSH), triiodothyronine (T3), and thyroxine (T4). A few studies have examined Ca^{2+} and cortisol.

4.9.3.1 Summary of Key Findings of the Effects on the Endocrine System from the 2006 Pb AQCD

The 2006 Pb AQCD (U.S. EPA, 2006b) reported that adult human endocrine processes impacted by occupational Pb exposure include thyroid hormone levels, changes in male sex hormone levels, as well as changes in the production of vitamin D (1,25-(OH)2D3). However, these effects were observed only with blood Pb levels exceeding 30–40 µg/dL and in cross-sectional studies with little or no consideration for potential confounding by factors such as sex, SES, nutritional status, BMI, smoking, comorbid conditions, and other occupational exposures. In addition, alterations in calcitropic hormones were found in children with blood Pb levels ranging from 10-120 µg/dL and in an opposite direction than that in Pb-exposed workers.

4.9.3.2 Recent Epidemiologic Studies

Recent epidemiologic studies have reported associations between indicators of exposure to Pb and thyroid hormone levels, in populations of children and in adults (with and without occupational Pb exposure); although results have not been consistent for a particular hormone. Further, the implications of these findings are limited because of the use of cross-sectional study design, lack of rigorous statistical analysis, and limited consideration for potential confounding factors. Inconsistent associations were found in a study that did consider potential confounding factors. Abdelouahab et al. (2008) examined an adult Canadian population characterized by high consumption of freshwater fish contaminated with Pb and other environmental pollutants. The median concurrent blood Pb level was 3.1 µg/dL for men and 1.7 µg/dL for women. The median blood Pb level for women was lower than the limit of detection (2.1 µg/dL), resulting in measurement error of blood Pb level and greater uncertainty in the results. In an analysis stratified by sex, TSH levels were negatively correlated with blood Pb in women with adjustment for age, smoking status, estro-progestative intake, total plasma lipids, and total blood selenium (Se). No associations with T3 and T4 levels were found in women. TSH, T3 and T4 levels were not correlated with blood Pb level in males, after adjustment for the same covariates (excluding hormone intake) plus pesticide exposure, corticoid medication, concurrent alcohol consumption, and occupational exposure to metals. Overall, the inconsistent associations and potential influence of other exposures did not strongly demonstrate an effect of Pb exposure.

Studies with less rigorous methods also did not clearly indicate an association between blood Pb level and a particular thyroid hormone. In a Kosovo, Yugoslavia population of pregnant women, higher pregnancy blood Pb levels were associated with lower free T4 levels during pregnancy among the 156 women living in a highly exposed town with a smelter and battery plant, but not among the 153 women living in a relatively unexposed nearby town (Lamb et al., 2008). The mid-pregnancy blood Pb levels were highly elevated in the industrial town compared to the unexposed town (mean [SD]: 20.56 [7.38] versus 5.60 [1.99] µg/dL). In 24 newborns delivered in Tokyo, Japan, neither TSH nor free T4 (sampled 4-6 days postpartum) was correlated with cord blood Pb level (mean: 0.67 µg/dL) (Iijima et al., 2007). Neither of these two studies considered potential confounding. Croes et al. (2009) examined the hormone levels in 1,679 adolescent girls and boys residing in nine study areas in the Flanders Region of Belgium, with varying exposures to multiple industrial pollutants including Pb. The median concurrent blood Pb level of the participants from the nine different study areas ranged from 1.6 to 2.8 µg/dL. Analyses only indicated differences in free T3, at the region or neighborhood level with adjustment for age, sex, recent disease, and BMI. No direct associations with blood Pb

level were analyzed, thus the results could be attributed to other factors that varied by location.

Contrasting results were found for free T4 in Pb-exposed workers. Dundar et al. (2006) examined associations between blood Pb levels and thyroid function in 42 male adolescent auto repair apprentice workers, with no history of prior disease and exposed long term to Pb (15-17 year-olds in the auto repair apprenticeship at least 1 year). Mean blood Pb level was higher in the auto repair workers compared to the 55 healthy unexposed control subjects (mean [SD]: 7.3 [2.92] versus 2.08 [1.24] µg/dL). Free T4 levels were significantly lower in the auto workers compared to the control group, which had no abnormal free T4 levels reported. In contrast, free T3 and TSH levels were comparable between auto workers and controls. Blood Pb level was negatively correlated with free T4 levels. In contrast, another study (Pekcici et al., 2010) found higher free T4 and TSH in adult auto mechanic or battery factory workers who were highly exposed to Pb (mean blood Pb: 71.1 µg/dL) compared to controls (mean blood Pb level: 0.2 µg/dL). Free T3 levels were similar between the two groups. The results from this study are likely not generalizable to the general public due to the high blood Pb levels of the exposed workers.

Previous findings for blood Pb-associated changes in serum vitamin D (1,25-(OH)2D3) in children were mixed. A recent study in New Jersey examined winter (December to March) and summer (July to September) seasonal changes in the associations between blood Pb level and serum 1,25-(OH)2D3 status, in 142 young, U.S. urban African-American or Hispanic children (ages 1-8 years, grouped by age [1-3 year-olds and 4-8 year-olds] and race/ethnicity) using a repeated measures design (Kemp et al., 2007). The percentage of 1-3 year-old African-American children (n = 49) with blood Pb levels ≥ 10 µg/dL increased from 12.2% in winter to 22.5% in summer. This large seasonal increase in blood Pb levels in these 1–3 year-old children was not accompanied by a significant increase in serum 1,25-(OH)2D3 concentrations. There was also a larger seasonal increase in blood Pb levels in 1-3 year-old children from both races combined (n = 78) (mean [SE]: 4.94 [0.45] µg/dL winter, 6.54 [0.82] µg/dL summer) than in 4-8 year-old children from both races combined (n = 64) (mean [SE]: 3.68 [0.31] µg/dL winter, 4.16 [0.36] µg/dL summer). However, no difference in seasonal 1,25-(OH)2D3 was observed in the 1-3 year-old children from both races combined. A larger winter to summer increase in blood Pb level was correlated with a larger seasonal increase in serum 1,25-(OH)2D3 in the 4-8 year-old children from both races combined and in the 4-8 year-old African American children (n = 42). In the 4-8 year-old children from both races combined, there was a winter to summer increase in 1,25-(OH)2D3 (mean [SE]: 25.3 [1.2] µg/L in winter versus 33.8 [1.1] µg/L in summer), which may account for the results in this older age group. Based on these results, the study authors concluded that

higher summertime increase in serum 1,25-(OH)2D3 levels in children between ages 4 and 8 years is most likely due to increased sunlight-induced vitamin D synthesis and may be a contributing factor to seasonal changes in blood Pb levels via changes in gastrointestinal absorption or release of Pb from bone.

Hypothalamic-pituitary-adrenal (HPA) axis function was examined in a prospective analysis of associations between prenatal maternal blood Pb levels (indicated by cord blood collected at delivery) or postnatal blood Pb levels (at mean [SD] age: 2.62 [1.2] years, data obtained from family physicians or state records), and saliva cortisol levels during a stress protocol in the children at age 9.5 years (Gump et al., 2008). For prenatal blood Pb, the children were divided into the following quartiles: [≤ 1], [1.1-1.4], [1.5-1.9], and [2.0-6.3] µg/dL. For postnatal blood Pb, the quartiles were: [1.5-2.8], [2.9-4.1], [4.2-5.4], and [5.5-13.1] µg/dL. With adjustment for potential confounding by SES-related factors, HOME score, pregnancy health, maternal substance abuse, blood Pb level was not associated with initial salivary cortisol levels in the children when they were older (9.5 years old), before the stress challenge protocol commenced. However, following an acute stressor, which comprised of submerging the dominant arm of each 9 year-old child for a minute in a gallon of one part ice to one part water, increasing prenatal and postnatal blood Pb levels were associated with statistically significant increases in salivary cortisol responses. Children in the 2nd, 3rd, and 4th prenatal blood Pb quartiles and in the 4th postnatal quartile had increased salivary cortisol responses compared to children in the 1st quartile. When blood Pb was treated as a continuous variable, regression analysis showed that both prenatal and postnatal blood Pb levels were associated salivary cortisol reactivity. While associations were found in children with blood Pb levels below 10 µg/dL, they could have been attributed to higher earlier childhood blood Pb levels of these children who were born in 1980-1990s.

4.9.3.3 Recent Toxicological Studies

Pb-associated changes in thyroid hormones also were found in animal studies. In a study examining the effects of Pb and Cd in adult cows reared in a polluted environment in India, Swarup et al. (2007) found significantly higher mean plasma T3 and T4 levels in cows living near Pb/Zn smelters (mean [SD] blood Pb: 86 [6] µg/dL) and near closed Pb/operational Zn smelters (mean [SD] blood Pb: 51 [9] µg/dL) when compared to cows in unpolluted areas (mean [SD] blood Pb: 7 [1] µg/dL). Regression analyses of the 269 cows showed a significant positive correlation between blood Pb levels and plasma T3 and T4 levels, whereas the correlation between blood Pb levels and plasma cortisol was not statistically significant. Mean plasma estradiol level was significantly higher in cows

near the closed Pb/operational Zn smelters compared to the control group of cows. Because of the Pb-Zn co-exposure, the effects cannot be attributed specifically to Pb.

Biswas and Ghosh (2006) investigated the effect of Pb treatment on adrenal and male gonadal functions in Wistar rats treated with Pb acetate (8.0 mg/kg via i.p. injection for 21 days, resulting blood Pb levels not reported). Pb treatment significantly increased adrenal steroidogenic enzyme activity and serum corticosterone levels. Accessory sex organ (prostate and seminal vesicle) weights were decreased in Pb-treated animals, whereas adrenal weights were increased. These effects were accompanied by a decrease in spermatogenesis and serum concentrations of testosterone, FSH, and LH and by an increase in the percent of spermatid degeneration. Supplementation with testosterone during the last 14 days of Pb treatment was observed to ameliorate these effects.

4.9.3.4 Summary of Endocrine Effects

Collective epidemiologic and toxicological evidence is inconsistent in demonstrating the effects of Pb exposure on male and female sex hormone levels (Sections 4.8.3 and 4.8.4) and vitamin D levels. Several epidemiologic studies have reported associations between indicators of Pb exposure and thyroid hormone levels in populations of children and adults without (Lamb et al., 2008) and with occupational Pb exposure (Dundar et al., 2006), although results have not been consistent for a particular hormone. Further, the implications of these findings are limited because of the cross-sectional study design, lack of rigorous statistical analysis, and limited consideration for potential confounding factors. Blood Pb level was positively correlated with plasma T3 and T4 levels in adult cows living near Pb-Zn smelters; however, the effects could not be attributed specifically to Pb exposure (Swarup et al., 2007).

In a prospective study of children in New York, who were challenged with an acute stressor, higher cord blood levels (as a reflection of prenatal maternal Pb blood level), or 2-year-old blood Pb levels, were associated with significant higher salivary cortisol in response to a stress challenge at age 9 years (Gump et al., 2008). While these associations were found with blood Pb levels <10 µg/dL, they could have been attributed to higher earlier childhood blood Pb levels of these children who were born in the 1980s and 1990s. Biswas and Ghosh (2006) found a Pb-induced increase in corticosterone in rats, albeit by i.p. Pb treatment. Cortisol and corticosterone are the major glucocorticoids in humans and rodent, respectively.

In conclusion, epidemiologic and toxicological evidence indicates Pb-associated endocrine effects such as thyroid hormones, cortisol, and vitamin D, although results are not consistent. Because of the lack of insufficient quantity and quality of studies, the

evidence is inadequate to determine if there is a causal relationship between Pb exposure and endocrine effects related to thyroid hormones, cortisol, and vitamin D.

4.9.4 Effects on Bone and Teeth

Primary effects on bone associated with Pb exposure or biomarker levels have included an increase in osteoporosis, increased frequencies of falls and fractures, changes in bone cell function as a result of replacement of bone calcium with Pb, and depression in early bone growth. Other effects include tooth loss and periodontitis. Mechanistic evidence from toxicological studies includes effects on cell proliferation, procollagen type I production, intracellular protein, and osteocalcin in human dental pulp cell cultures.

4.9.4.1 Summary of Key Findings of the Effects on Bone and Teeth from the 2006 Pb AQCD

The 2006 Pb AQCD reported many effects on bone and some in teeth in animals following Pb exposure. Exposure of animals to Pb during gestation and the immediate postnatal period was reported to significantly depress early bone growth with the effects showing concentration-dependent trends. In mature animals, long-term Pb exposure (up to one year), along with poor nutrition (low calcium) reduced bone growth as well as bone density. Systemic effects of Pb exposure included disruption in bone mineralization during growth, alteration in bone cell differentiation and function due to alterations in plasma levels of growth hormones and calcitropic hormones such as 1,25-$[OH]_2$D3 and impact on Ca^{2+}- binding proteins and increases in Ca^{2+} and phosphorus concentrations in the bloodstream. Bone cell cultures exposed to Pb had altered vitamin D-stimulated production of osteocalcin accompanied by inhibited secretion of bone-related proteins such as osteonectin and collagen. In addition, Pb exposure caused suppression in bone cell proliferation most likely due to interference from factors such as growth hormone (GH), epidermal growth factor (EGF), transforming growth factor-beta 1 (TGF-β1), and parathyroid hormone-related protein (PTHrP).

As in bone, Pb exposure was found to easily substitute for Ca^{2+} in the teeth and was taken up and incorporated into developing teeth in experimental animals. Since teeth do not undergo remodeling like bone does during growth, most of the Pb in the teeth remains in a state of permanent storage. High dose Pb exposure to animals (30 mg/kg body weight) was found to induce the formation of a “Pb line” that is visible in both the enamel and dentin and is localized in areas of recently formed tooth structure. Areas of mineralization were easily evident in the enamel and the dentin within these “Pb lines.” Pb has also been

shown to decrease cell proliferation, procollagen type I production, intracellular protein, and osteocalcin in human dental pulp cell cultures. Adult rats exposed to Pb have exhibited an inhibition of the posteruptive enamel proteinases, delayed teeth eruption times, as well as a decrease in microhardness of surface enamel. Pb was reported to be widely dispersed and incorporated into developing apatite crystal during enamel formation process; however, post formation, Pb was reported to be capable of entering and concentrating in specific enamel areas which were Ca^{2+}-deficient. The 2006 Pb AQCD (U.S. EPA, 2006b) also reported that a number of animal studies and a few epidemiologic studies each suggested that Pb is a caries-promoting element. The strongest epidemiologic evidence comprised associations between concurrent blood Pb level and dental caries in an NHANES analysis of thousands of children that adjusted for age, sex, race/ethnicity, poverty to income ratio, exposure to cigarette smoke, geographic region, head of household education, carbohydrate and calcium intake, and frequency of dental visits. Other effects found in humans included bone disease (e.g., Paget's disease); however, the evidence was provided by occupational or case-control studies.

4.9.4.2 Recent Toxicological and Epidemiologic Studies

Consistent with evidence reported in the 2006 Pb AQCD, recent studies have found associations between Pb exposure or biomarker levels and effects in bones of humans and animals. The association between blood Pb levels and lower bone mineral density was examined in several epidemiologic studies. Prospective evidence was provided by Khalil et al. (2008) in 533 older women aged 65-87 years with a mean (SD) blood Pb levels of 5.3 (2.3) µg/dL. Base line bone mineral density was measured in 1986-1988 (calcaneus), measured again in 1988-1990 (total hip and femoral neck), and again in 1993-1994 (calcaneus, total hip and femoral neck), while blood Pb levels were measured in between the second and last bone analyses (during 1990-1991; and categorized as low [n = 122, ≤ 3 µg/dL], medium [n = 332, 4-7 µg/dL], and high [n = 79, ≥ 8 µg/dL] Pb blood levels [range: 1-21 µg/dL]). Information on falls and fractures was collected every 4 months, starting after the initial enrollment (1986-1988) and continuing for more than 10 years. The bone mineral density at the last measurement (1993-1994) was 7% lower in the total hip ($p < 0.02$) and 5% lower in the femoral neck ($p < 0.03$) in the high blood Pb level group (≥ 8 µg/dL) compared to the low blood Pb level group (≤ 3 µg/dL). A concentration-dependent relationship was found for total hip and femoral neck bone mineral density across the three blood Pb level groups. In addition, total hip, femoral neck, and calcaneus bone loss was observed to be greater in the medium (blood Pb level: 4-7 µg/dL) and high blood Pb level groups compared to the low Pb level group, with a statistically significant trend found for calcaneus bone loss. Compared to the low blood

Pb level group, women in the high blood Pb level group had an increased risk of non-spine fracture (10.5 year interview follow-up), and women with medium or high blood Pb levels had a higher risk of falls (4 year follow-up) with adjustment for age, clinic, BMI, weight change between visits, smoking, chair stands (were able to stand up five times from a chair, without using the arms of the chair), fracture history, estrogen use, and baseline (1986-1988) bone mineral density. Nutritional factors were not considered. The increased risk of lower bone density and falls leading to osteoporosis-related fractures associated with blood Pb levels (>4 µg/dL) are likely influenced by higher past Pb exposures of these women.

Supporting evidence was provided by cross-sectional epidemiologic studies, although the direction of the association and the magnitude, timing, frequency, and duration of Pb exposure that contributed to the observed associations are uncertain. Further, most studies did not consider potential confounding by nutritional factors. A large NHANES II analysis of 8,654 adults ≥ 50 years of age (Campbell and Auinger, 2007) was stratified by non-Hispanic white men (mean blood Pb level: 4.9 [range: 0.7 to 48.1] µg/dL), non-Hispanic white women (mean blood Pb level: 3.6 [range: 0.7 to 28.7] µg/dL), African-American men (mean blood Pb level: 7.7 [range: 0.7 to 52.9] µg/dL), and African-American women (mean blood Pb level: 4.5 [range: 0.7 to 23.3] µg/dL). Analyses of covariance considered potential confounding by age, race, sex, BMI, menopausal status, tobacco use, alcohol use, physical activity, Ca^{2+} intake, chronic medical conditions, certain medication use, and SES. Non-Hispanic white men ($n = 1,693$, $p < 0.05$) and women ($n = 1,754$, $p < 0.10$) in the highest tertile of concurrent blood Pb level had lower mean total hip bone mineral density than non-Hispanic white men and women in the lowest tertile of blood Pb levels (actual concentrations not reported). Smaller differences were observed in African-American men and women (possibly due to the smaller sample sizes ($n = 613$, and 629, respectively). No association was observed between blood Pb levels and osteoporotic fractures in either sex or any race/ethnicity group.

Similar observations were made by Sun et al. (2008a) in 155 males and 37 females in China who were occupationally-exposed to Pb (mean blood Pb: 20.22 and 15.5 µg/dL, respectively). In analyses (including all workers, [plus 36 male and 21 female unexposed controls] stratified into groups according to blood Pb and urinary Pb levels), groups with urinary Pb levels ≥ 5 µg Pb/g of creatinine had lower ($p < 0.01$) bone mineral density compared to groups with lower urinary Pb in each sex. Prevalence of osteoporosis increased with increasing blood Pb in a linear manner. In contrast, a significant association was observed between blood Pb level and bone mineral density, but only in men with blood Pb levels >30 µg/dL. Prevalence of osteoporosis increased significantly

with increasing blood Pb in a linear manner. Results were not adjusted for potential confounding factors, including other occupational exposures.

Cross-sectional epidemiologic studies also found associations between concurrent blood Pb level and biological markers of bone turnover. Among 329 male (mean age: 65 years, median blood Pb level: 2.2 µg/dL) and 342 female (mean age: 62 years, median blood Pb level: 1.9 µg/dL) adults in North Carolina, Nelson et al. (2011) found in women that higher blood Pb level was associated with biochemical biomarkers of joint tissue metabolism and turnover, specifically higher urinary N-terminal cross-linked telopeptide of type I collagen (uNTX-I, a marker of bone resorption/turnover) and urinary C-terminal cross-linked telopeptide fragments of type II collagen (uCTX-II, a marker associated with the progression of radiographic knee and hip osteoarthritis) after adjusting for age, BMI, race, and smoking status. In adjusted analyses of men, higher blood Pb level was associated with higher uCTX-II, COMP (cartilage oligomeric matrix protein), and type II collagen cleavage neoepitope / C-propeptide of type II procollagen (C2C:CPII) ratio (an indication of the balance between cartilage collagen degradation and synthesis). In women, a weaker association was found for COMP, a cartilage biomarker related to osteroarthritis. The results indicated that blood Pb level is associated with bone turnover and mineralized cartilage turnover in women, and with non-mineralized cartilage turnover in men.

Similarly, Machida et al. (2009) investigated bone matrix turnover in Japanese women farmers, and how it is related to age-related menopause status and blood Pb level. Perimenopausal women (n = 319 [age range: 49 to 55 years]) had higher geometric mean blood Pb level (2.0 µg/dL) than the other 3 groups did: premenopausal women (n = 261 [age range: 35 to 48 years], blood Pb level: 1.6 µg/dL), younger postmenopausal women (n = 397 [age range: 56 to 65 years], blood Pb level: 1.8 µg/dL), or older postmenopausal women (n = 248 [age range: 66 to 75 years], blood Pb level: 1.7 µg/dL). In a model that simultaneously included bone-mineral density, uNTX-I, osteocalcin, and age, higher blood Pb levels were positively associated with bone mineral density, uNTX-I, and osteocalcin (all $p < 0.01$). In perimenopausal women, higher blood Pb level was predicted most strongly by higher osteocalcin levels. Age was positively associated with higher blood Pb levels in perimenopausal women only. Associations also were reported for bone-specific ALP in unadjusted analyses.

To characterize mechanisms underlying the effects of Pb on bone, Jang et al. (2008) studied the effect of Pb exposure on Ca^{2+}-release activated Ca^{2+}-influx (CRACI) using cultures of human fetal osteoblast-like (hFOB 1.19) cells (OLCs) in vitro. When cells were incubated with 1,000 or 3,000 µM Pb in the culture medium, a concentration-dependent decrease in CRACI was observed, as was a concentration-dependent increase

in the influx of Pb into the human OLCs. These results suggest that Pb inhibits the measurable influx of Ca^{2+} upon re-addition of Ca^{2+}, which in turn, results in an influx of Pb into the OLCs.

Studies have found inconsistent associations between higher blood Pb levels and reduced growth in children; however, Zuscik et al. (2007) hypothesized that Pb may alter growth by altering chondrogenic commitment of mesenchymal cells and by affecting various signaling pathways. Exposure of stage E11.5 murine limb bud mesenchymal cells (MSCs) to 1 µM Pb in vitro caused increased basal and TGF-β/BMP induction of chondrogenesis, which was accompanied by nodule formation and upregulation of Sox-9, type 2 collagen, and aggrecan, which are all key markers of chondrogenesis. Enhanced chondrogenesis during induced ectopic bone formation also was found in mice that had been pre-exposed to Pb acetate for six weeks via drinking water (55 or 233 ppm, [previously shown to correspond to 14 or 40 µg/dL blood Pb level, respectively]). MSCs exposed to Pb in vitro exhibited an increase in TGF-β, but BMP-2 signaling was inhibited. Pb also induced NF-κB and inhibited AP-1 signaling. These results suggested that the chondrogenesis induced by Pb exposure most likely involved modulation and integration of multiple signaling pathways, including TGF-β, BMP, AP-1, and NF-κB.

Effects of Pb exposure on teeth were examined in a few recent cross-sectional epidemiologic studies. A subset of the U.S. NHANES III (1988-1994) population was selected for a large periodontitis versus Pb blood level study of both men (n = 2,500) and women (n = 2,399), 30-55 years-old, that considered potential confounding by a large set of factors, including nutritional status (Saraiva et al., 2007). Compared to individuals with a concurrent blood Pb level of <3 µg/dL, the prevalence ratios of periodontitis were 1.70 (95% CI: 1.02, 2.85) for men with concurrent blood Pb level of >7 µg/dL and 3.80 (95% CI: 1.66, 8.73) for women with concurrent blood Pb level >7 µg/dL. These results were adjusted for age, NHANES III phase, cotinine levels, poverty to income ratio, race/ethnicity, education, bone mineral density, diabetes, Ca^{2+} intake, dental visits, and menopause status in women.

Arora et al. (2009) examined the association between blood and bone Pb level and the loss of natural teeth, in 333 men (age range: 50 to 94 years) from a subset of the Veterans Affairs Normative Aging Study (NAS). Tooth loss was ascertained as the number of teeth present during a dental assessment, and was categorized into three groups: 0 missing teeth (n = 44), 1-8 missing teeth (n = 164), or ≥ 9 missing teeth (n = 125). Men with ≥ 9 teeth missing had significantly higher tibia and patella Pb concentrations (measured within 3 years of dental assessment) compared to those with no tooth loss. Men with the highest tibia Pb concentrations (>23 µg/g) had higher odds of tooth loss (OR: 3.03 [95% CI: 1.60, 5.75]) compared to men with tibia Pb levels ≤ 15 µg/g. Men with the highest

patella Pb levels (>36 µg/g) also had higher odds of tooth loss (≥ 9 missing teeth versus 0-8 missing teeth; or ≥ 1 missing teeth versus 0 missing teeth: OR: 2.41 [95% CI: 1.30, 4.49]) compared to men with patella Pb levels ≤ 22.0 µg/g. Men with tibia Pb levels 16-23 µg/g, and men with patella Pb levels 23-36 µg/g also had elevated odds of tooth loss. Results were adjusted for age, education, smoking status, pack-years of smoking, and diabetes, but nutritional factors were not considered. Tooth loss was not associated with higher blood Pb levels which were measured within 3 years of dental assessment [see Hu et al. (1996b) for details of the blood Pb analysis]. This indicates that long-term cumulative exposure to Pb is associated with increased odds of tooth loss. However, because the timing of tooth loss was not ascertained, and concurrent bone Pb levels may represent exposures after tooth loss occurred, the directionality of effects is uncertain.

4.9.4.3 Summary of Effects on Bone and Teeth

A few studies have indicated associations of Pb exposure or Pb biomarker levels with bone disease (e.g., Paget's disease); however, the implications are limited by examination of Pb-exposed workers or recruitment of individuals based on bone disease status (i.e., case-control). Numerous epidemiologic studies indicated an association between higher Pb biomarker levels and lower bone density in adults. Prospective evidence was provided by a study of elderly women (65-87 years-old), in which higher blood Pb levels (> 4 µg/dL vs. < 3 µg/dL) were associated with lower bone density measured after 2-4 years and greater risk of falls and osteoporosis-related fractures (Khalil et al., 2008). Cross-sectional epidemiologic associations between higher blood Pb levels and lower bone mineral density were found in adults without (Campbell and Auinger, 2007) and with occupational Pb exposure (Sun et al., 2008a). Cross-sectional studies also indicated associations between higher blood Pb levels and biochemical biomarkers of higher bone turnover in elderly populations (Nelson et al., 2011; Machida et al., 2009). In the cross-sectional epidemiologic evidence, it is difficult to determine whether an increase in blood Pb level results from lower bone density or from higher bone turnover, and whether either of these effects lead to a greater release of Pb from bone into the bloodstream. Except for Sun et al. (2008a), studies adjusted for several potentially important confounding factors, including age, BMI, and smoking. However, studies did not consider nutritional status, which could affect the release of Pb from bone to blood. To support the direction and independent effects of Pb on bone development, toxicological studies have found Pb-induced (gestational and postnatal) decreases in bone growth in juvenile animals. Further, these toxicological studies have characterized potential modes of action, by showing Pb-induced decreases in bone mineralization and bone cell

differentiation, inhibition of CRACI, and alterations in signaling pathways involved in skeletal development (Jang et al., 2008; Zuscik et al., 2007).

Epidemiologic studies have found associations between blood Pb levels and effects on teeth. Large NHANES analyses adjusted for several potentially important confounding factors including age, SES-related factors, and nutritional factors, and found associations between concurrent blood Pb level and dental caries in children (Moss et al., 1999) and periodontitis in adults (Saraiva et al., 2007). Higher patella and tibia Pb levels were associated with tooth loss in NAS men (Arora et al., 2009). The results for blood Pb and bone Pb levels in adults indicate that long-term, cumulative exposure to Pb exposure is associated with negative effects on teeth. This epidemiologic evidence was based on cross-sectional study design analyses, which precludes conclusions about the directionality of effects. However, these findings are supported by toxicological evidence in animals for Pb-induced increases in Pb uptake into teeth; and decreases in cell proliferation, procollagen type I production, intracellular protein, and osteocalcin in cells exposed to Pb in vitro. Despite evidence for associations between Pb exposure and effects in bone and teeth at relatively low concurrent blood Pb levels, these outcomes were most often examined in older adults that have been exposed to higher levels of Pb earlier in life. Therefore, uncertainty still remains concerning the Pb exposure level, timing, frequency, and duration that contribute to the observed associations.

However, the small body of epidemiologic evidence showing associations between Pb biomarker levels and various bone and teeth effects after adjusting for potential confounding by age, SES-related factors, and nutritional factors, plus the supporting toxicological evidence, is sufficient to conclude that a causal relationship is likely to exist between Pb exposure and effects on bone and teeth.

4.9.5 Effects on Ocular Health

Ocular effects most commonly associated with exposure to Pb include formation of cataracts, impaired vision, retinal edema, and retinal stippling.

4.9.5.1 Summary of Key Findings of the Effects on Ocular Health from the 2006 Pb AQCD

The 2006 Pb AQCD stated that various changes in the visual system were observed with Pb poisoning including retinal stippling and edema, cataracts, ocular muscle paralysis and impaired vision. Maternal prenatal blood Pb levels in the range of 10.5 to 32.5 μg/dL during the first trimester were associated with supernormal retinal electroretinograms

(ERGs) in children at age 7-10 years (Section 4.3.6.2). Cataracts were noted in middle-aged men with tibia bone Pb levels of 31-126 µg/g.

4.9.5.2 Recent Toxicological and Epidemiologic Studies

The recent cross-sectional epidemiologic studies of ocular effects in adults did not produce clear evidence, and each was limited by the lack of rigorous statistical analysis and lack of consideration for potential confounding. Erie et al. (2009) measured Pb and Cd in retinal tissue from 25 eye donors with age-related macular degeneration (cases) and 36 normal control donors. Pb, but not Cd, concentration was significantly elevated in the neural retina tissue of the 25 donors with macular degeneration (50 eyes; median [IQR]: 12.0 [8-18] ng/g Pb) versus normal control donors (72 eyes; median [IQR]: 8.0 [0-11] ng/g Pb). Neither of these heavy metals was significantly elevated in the retinal pigment epithelium (RPE)/choroid complex in donors with macular degeneration over normal controls. Mosad et al. (2010) compared Pb, Cd, vitamin C, vitamin E, and beta carotene blood levels between 45 middle-aged male smokers and nonsmokers with cataracts. Blood Pb levels were elevated ($p < 0.0001$) in 15 light (mean [SD]: 14.5 [0.41] µg/dL), 15 moderate (14.5 [0.41] µg/dL), and 15 heavy smokers (18.7 [1.24] µg/dL) compared to 15 nonsmokers (12.2 [0.21] µg/dL). Similar associations were observed for Cd blood levels and lens concentrations. There was no direct analysis of the association between Pb blood level or lens Pb concentration and the severity of cataracts.

Recent animal studies have observed Pb-induced retinal progenitor cell proliferation and neurogenesis (Section 4.3.6.2). An in vitro study found increased opacity of rat (age 4-6 weeks) lens exposed to 1 µM Pb nitrate with or without secondary oxidative challenge after 5-8 days but not after 3 days (Neal et al., 2010b). Thus, short-term Pb exposure did not induce osmotic swelling or lens shrinkage. Following a 5-day exposure, approximately 30% of the Pb-exposed lenses displayed "definite cataracts" compared to only 5% of control lenses. By culture day 8, 100% of the Pb-exposed lenses were described either as having point opacities or definite cataracts, while only approximately 14% of control lenses displayed these characteristics, indicating that prolonged exposure of lenses to Pb induced an accelerated formation of opacity/cataract compared to unexposed lenses. Pb-exposed lenses cleared the media of hydrogen peroxide more rapidly than did control lenses, potentially due to increased CAT activity. Exposure to hydrogen peroxide resulted in total (100%) opacity in Pb-exposed lenses at culture day 7, compared ~20% in control cells. Exposure to Pb additionally altered epithelial nutrient transport and lens histology, relative to that in controls.

In summary, a prospective epidemiologic study indicated associations between prenatal maternal blood Pb levels of 10.5-32.5 µg/dL during the first trimester and supernormal retinal ERGs in children ages 7-10 years [Section 4.3.6.2, Rothenberg et al. (2002b)]. However, the biological relevance of supernormal ERGs is uncertain. Evidence in adults for associations between eye tissue Pb levels and macular degeneration (Mosad et al., 2010) and cataracts in adults (Erie et al., 2009) is limited by weak statistical methods and lack of consideration for potential confounding to warrant conclusions. Toxicology studies have reported Pb-induced retinal progenitor cell proliferation, retinal ERGs, and lens opacity (Section 4.3.6.2). Because of the insufficient quantity and quality of studies in the cumulative body of evidence, the evidence is inadequate to determine a causal relationship between Pb exposure and ocular effects.

4.9.6 Effects on the Respiratory System

Blood Pb level has been associated with asthma and allergy in children in prospective and cross-sectional epidemiologic studies (Section 4.6.5.2). As described in Section 4.2.4, Pb exposure has been shown to induce the generation of ROS. ROS are implicated in mediating increases in bronchial responsiveness and activating neural reflexes, leading to decrements in lung function. Collectively, studies investigating these airway responses in asthma-free populations are limited in number, lack rigorous statistical analysis, and collectively do not provide strong evidence of an association with blood Pb level (Section 4.6.5.3). Collectively, panel and time-series epidemiologic studies demonstrate associations between short-term increases in ambient air Pb (measured in $PM_{2.5}$ or PM_{10} air samples), and decreases in lung function and increases in respiratory symptoms, and asthma hospitalizations in children but not adults (Section 4.6.5.3). Toxicological studies have found pulmonary inflammation induced by concentrated ambient air particles (CAPs) in which Pb was one of the numerous components (Wei et al., 2011; Duvall et al., 2008; Godleski et al., 2002; Saldiva et al., 2002). Despite this evidence for respiratory effects related to air-Pb concentrations, the limitations of air-Pb studies, including the limited data on the size distribution of Pb-PM (Section 2.5.3), the uncertain relationships of Pb-PM_{10} and Pb-$PM_{2.5}$ with blood Pb levels, and the lack of adjustment for other correlated PM chemical components preclude firm conclusions about air Pb-associated respiratory effects. Because of the insufficient quantity and quality of studies in the cumulative body of evidence, the evidence is inadequate to determine a causal relationship between Pb exposure and respiratory effects in populations without asthma.

4.10 Cancer

Previous Pb AQCDs have demonstrated that Pb is a well-established animal carcinogen. Oral Pb acetate exposure to male and female rodents has consistently been shown to be a kidney carcinogen in multiple separate studies, inducing adenocarcinomas and adenomas after chronic exposure. Developmental Pb acetate exposure also induced kidney tumors in offspring whose dams received Pb acetate in drinking water during pregnancy and lactation. Gliomas of the brain have also been reported after oral Pb exposure. These rodent toxicological studies have been conducted at high doses of Pb and have shown that Pb is an animal carcinogen. Because of this strong body of historical data, the 2006 Pb AQCD, in describing the evidence reviewed therein, states, "limited tumorigenesis studies have been conducted in animal models and the focus has been more on the mechanism of neoplasia…and possible immunomodulatory effects of Pb in the promotion of cancer." More recent studies have focused on co-administration of Pb with known carcinogens or modifiers such that lifestage, diet, and mechanism of action can be better understood.

The previous epidemiologic studies included in the 2006 Pb AQCD (U.S. EPA, 2006b) "provide[d] only very limited evidence suggestive of Pb exposure associations with carcinogenic or genotoxic effects in humans," and the studies were summarized as follows:

> "The epidemiologic data …suggest a relationship between Pb exposure and cancers of the lung and the stomach… Studies of genotoxicity consistently link Pb-exposed populations with DNA damage and micronuclei formation, although less consistently with chromosomal aberrations."

The International Agency for Research on Cancer (IARC) classified inorganic Pb compounds as probable human carcinogens (Group 2A of IARC classification) based on sufficient evidence in animal studies (evidence in human studies was limited), and organic Pb compounds as not classifiable (Group 3 of IARC classifications) (IARC, 2006a; Rousseau et al., 2005). Additionally, the National Toxicology Program (NTP) listed Pb and Pb compounds as "reasonably anticipated to be human carcinogens" (NTP, 2011). The typical cancer bioassays employed by IARC or NTP as evidence of Pb-induced carcinogenicity used rodents that were continuously exposed to Pb acetate in chow or drinking water for 18 months to two years in duration. These two year cancer bioassays and the doses administered are typical of cancer bioassays used with other chemicals.

In the following sections, recent epidemiologic and toxicological studies, published since the 2006 Pb AQCD, regarding Pb and cancer mortality and incidence are examined. In addition, recent studies of Pb exposure associated with DNA and cellular damage, as well

as epigenetic effects, are summarized. When the information is available, the form of the Pb compound under study (e.g., inorganic, organic) is indicated. In epidemiologic studies, various biological indicators of Pb exposure are used including Pb measured in blood and bone. The biological indicators of Pb associated with cancer-related endpoints are considered in drawing conclusions about potentially important levels and timing of Pb exposure. Bone Pb is indicative of cumulative Pb exposure. Blood Pb can represent more recent exposure, but because it can also represent remobilized Pb occurring during times of bone remodeling, blood Pb level may also be an indicator of long-term Pb exposure in adults. More detailed discussion of these measures is given in Section 3.3.5. Toxicological studies report exposure or administered dose. Details of the recent epidemiologic and toxicological studies follow.

4.10.1 Cancer Incidence and Mortality

Recent studies have included epidemiologic evaluations of the associations between Pb exposure and both specific cancers (such as lung cancer and brain cancer), and overall cancer (cancer of any type). Table 4-49 provides an overview of the study characteristics and results for the epidemiologic studies that reported effect estimates. The studies in the text and table are grouped according to study design and methodological strength. This section also evaluates toxicological evidence on the potential carcinogenicity of Pb.

Table 4-49 Summary of recent epidemiologic studies of cancer incidence and overall cancer mortality.

Reference (In order of appearance in text)	Study Location	Cancer Outcome	Study Population	Methodological Details	Measure of Pb Exposure/ Concentration Data	Adjusted Effect Estimates	Potential confounders adjusted for in analysis
Overall Cancer Mortality:							
Menke et al. (2006)	Multiple U.S. locations	Overall cancer mortality	NHANES III cohort with Blood Pb measures in 1988-1994 Follow-up: At least 12 years Blood Pb <10 µg/dL N=13,946 N for cancer mortality = 411	Cohort study using Cox regression and other techniques	Blood Pb at baseline Geometric mean 2.58 µg/dL Tertile 1: <1.93 µg/dL Tertile 2: 1.94-3.62 µg/dL Tertile 3: ≥ 3.63 µg/dL	HR (95% CI): Tertile 1: 1.00 Tertile 2: 0.72 (0.46, 1.12) Tertile 3: 1.10 (0.82, 1.47)	Age, race/ethnicity, sex, diabetes mellitus, body mass index, current or former smoking, alcohol consumption, physical activity, low income, CRP, total cholesterol, high school education, urban residence, postmenopausal status, hypertension, and level of kidney function

Table 4-49 (Continued): Summary of recent epidemiologic studies of cancer incidence and overall cancer mortality.

Reference (In order of appearance in text)	**Study Location**	**Cancer Outcome**	**Study Population**	**Methodological Details**	**Measure of Pb Exposure/ Concentration Data**	**Adjusted Effect Estimates**	**Potential confounders adjusted for in analysis**
Schober et al. (2006)	Multiple U.S. locations	Overall cancer mortality	NHANES III cohort At least 40 years of age Median follow-up period for this study: 8.6 yr N= 9,757 N for cancer mortality = 543	Cohort study using Cox proportional hazard regression analysis and other techniques	Blood Pb at baseline Blood Pb<5 µg/dL: 67.7% Blood Pb 5-9 µg/dL : 26.0% Blood Pb≥10 µg/dL: 6.3%	RR (95% CI): Blood Pb<5 µg/dL: 1.00 Blood Pb 5-9 µg/dL: 1.44 (1.12, 1.86) Blood Pb≥ 10 µg/dL: 1.69 (1.14, 2.52) Note: Modification by age assessed and associations varied slightly	Sex, race/ethnicity, education, and smoking status Age used as time-scale in models Additional covariates considered but not included: Census region and urban status of residence, alcohol intake

Table 4-49 (Continued): Summary of recent epidemiologic studies of cancer incidence and overall cancer mortality.

Reference (In order of appearance in text)	Study Location	Cancer Outcome	Study Population	Methodological Details	Measure of Pb Exposure/ Concentration Data	Adjusted Effect Estimates	Potential confounders adjusted for in analysis
Weisskopf et al. (2009)	Boston, MA area	Overall cancer mortality	Normative Aging Study (NAS) Included men only (mostly white) Mean follow-up period for this study: 8.9 yr Blood Pb measures available N=1038 N for cancer mortality=85 Bone Pb measures available N=727 N for cancer mortality=57	Cohort study using Cox proportional hazards	Blood Pb at baseline (µg/dL) Mean (SD): 5.6 (3.4) Tertile 1 of Blood Pb: <4 Tertile 2 of Blood Pb: 4-6 Tertile 3 of Blood Pb: >6 Patella Pb at baseline (µg/g) Tertile 1 of patella Pb: <22 Tertile 2 of patella Pb: 22-35 Tertile 3 of patella Pb: >35	HR (95% CI): Blood Pb Tertile 1: 1.00 Blood Pb Tertile 2: 1.03 (0.42, 2.55) Blood Pb Tertile 3: 0.53 (0.20, 1.39) Patella Pb Tertile 1: 1.00 Patella Pb Tertile 2: 0.82 (0.26, 2.59) Patella Pb Tertile 3: 0.32 (0.08, 1.35)	Age, smoking, and education Additional covariates considered but not included: alcohol intake, physical activity, body mass index, total cholesterol, serum high-density lipoprotein, diabetes mellitus, race, and hypertension
Khalil et al. (2009b)	Baltimore, MD, and Monongahela Valley, PA	Overall cancer mortality	Subgroup of the Study of Osteoporotic Fractures cohort Included white women aged 65-87; Follow-up: 12 yr (+/- 3 yr) N=533 N for cancer mortality=38	Cohort study using Cox proportional hazards regression analysis and other techniques	Blood Pb at baseline Mean (SD) 5.3 (2.3) µg/dL	HR (95% CI): Blood Pb<8 µg/dL: 1.00 Blood Pb≥ 8 µg/dL: 1.64 (0.73, 3.71)	Age, clinic, body mass index, education, smoking, alcohol intake, estrogen use, hypertension, walking for exercise, diabetes, and total hip bone mineral density

Table 4-49 (Continued): Summary of recent epidemiologic studies of cancer incidence and overall cancer mortality.

Reference (In order of appearance in text)	Study Location	Cancer Outcome	Study Population	Methodological Details	Measure of Pb Exposure/ Concentration Data	Adjusted Effect Estimates	Potential confounders adjusted for in analysis
Overall Cancer Incidence:							
Absalon and Slesak (2010)	Silesia province, Poland	Overall cancer incidence	Children living in this province at least five years N = not specified	Ecologic analysis using correlations.	Pb-related air pollution measures Concentrations: NA	Reported correlations between changes in Pb and cancer incidence – no/low correlations observed (correlation coefficients between -0.3 and 0.2)	None specified Examined correlation by sex, no difference reported
Obhodas et al. (2007)	Island of Krk, Croatia	Incidence rates for neoplasms	Individuals living in the Island of Krk from 1997-2001 N= 1,940	Cross-sectional study using correlations and linear regression	Soil and vegetation samples, household potable water samples, children's hair samples Concentrations: NA	No association observed between Pb in the samples and incidence of neoplasm (numerical results not provided)	None specified
Mendy et al. (2012)	Multiple U.S. locations	Incidence of cancer or "malignancy of any kind"	2007-2008 NHANES cohort – at least 20 years of age N= 1,857	Cross-sectional study using logistic regression	Concurrently measured creatinine-adjusted urinary Pb Geometric mean for creatinine-adjusted urinary Pb marker: 0.59 µg/g (95% CI: 0.57, 0.61)	OR (95% CI): Greater than log-transformed mean creatinine-adjusted urinary Pb level compared to less than log-transformed mean creatinine-adjusted urinary Pb level: 0.76 (0.44, 1.33)	Age, sex, race/ethnicity, education level, ratio family income to poverty, alcohol consumption, cigarette smoking, and other heavy metals

Table 4-49 (Continued): Summary of recent epidemiologic studies of cancer incidence and overall cancer mortality.

Reference (In order of appearance in text)	Study Location	Cancer Outcome	Study Population	Methodological Details	Measure of Pb Exposure/ Concentration Data	Adjusted Effect Estimates	Potential confounders adjusted for in analysis
Lung Cancer:							
Lundstrom et al. (2006)	Sweden	Lung cancer (incidence and mortality)	Male Pb smelter workers first employed for ≥ 3 months between 1928 and 1979 Followed up for mortality from 1955 -1987 N=187 N lung cancer=46	Nested case-referent study using conditional logistic regression	Median peak blood Pb level Cases: 49.7 µg/dL Controls: 55.9 µg/dL Median number of years with at least one blood sample obtained Cases: 4.5 yr, Controls: 6.0 yr Median cumulative blood Pb index (sum of annual blood Pb Level) Cases: 186 µg/dL Controls: 246 µg/dL	OR (95% CI): Median peak blood Pb Level: 1.00 (0.71, 1.42) per 10 µg/dL Median number of years with at least one blood sample obtained: 0.96 (0.91, 1.02) per year Median cumulative Blood Pb index: 1.00 (0.98, 1.01) per 10 µg/dL Note: similar results were observed when restricted to smokers only	Matched by age Adjusted for smoking and As exposure

Table 4-49 (Continued): Summary of recent epidemiologic studies of cancer incidence and overall cancer mortality.

Reference (In order of appearance in text)	**Study Location**	**Cancer Outcome**	**Study Population**	**Methodological Details**	**Measure of Pb Exposure/ Concentration Data**	**Adjusted Effect Estimates**	**Potential confounders adjusted for in analysis**
Jones et al. (2007)	Humberside, U.K.	Lung cancer mortality	Male tin smelter employees N=1,462	Cohort study using Poisson regression	Personnel record cards and air sampling conducted from 1972-1991 Three exposure scenarios determined for working lifetime cumulative exposure – all have similar medians of approximately 2 mg/m^3 per yr Mean ~2.0 mg/m^3 per yr	RR (90% CI) for Pb exposure weighted by age and time since exposure: 1.54 (1.14, 2.08) Note: Similar results for other exposure determination scenarios.	Not specified

Table 4-49 (Continued): Summary of recent epidemiologic studies of cancer incidence and overall cancer mortality.

Reference (In order of appearance in text)	Study Location	Cancer Outcome	Study Population	Methodological Details	Measure of Pb Exposure/ Concentration Data	Adjusted Effect Estimates	Potential confounders adjusted for in analysis
Rousseau et al. (2007)	Montreal, Canada	Lung cancer incidence	Men aged 35-79 N population based controls= ranged from 271 to 471 depending on exposure of interest N controls with other cancers= ranged from 737 to 1203 depending on exposure of interest N lung cancer=ranged from 433 to 751 depending on exposure of interest	Population-based case-control study using unconditional logistic regression	Interview of job history and exposure matrix Ever exposed to: Organic Pb 3.0% Inorganic Pb 17.0% Pb in gasoline emissions 38.6%	OR (95% CI) for any versus no exposure Organic Pb exposure: 1.3 (0.5, 3.1) Inorganic Pb exposure: 1.1 (0.7, 1.7) Pb in gasoline emissions exposure: 0.8 (0.6, 1.1) Note: results are for comparisons using population-based controls; results for controls with other types of cancers were similar	Age, family income, cultural origin, proxy status, ever exposure to asbestos, silica, As, Cd, and chromium (VI)

Table 4-49 (Continued): Summary of recent epidemiologic studies of cancer incidence and overall cancer mortality.

Reference (In order of appearance in text)	Study Location	Cancer Outcome	Study Population	Methodological Details	Measure of Pb Exposure/ Concentration Data	Adjusted Effect Estimates	Potential confounders adjusted for in analysis
Brain Cancer:							
van Wijngaarden and Dosemeci (2006)	Multiple U.S locations	Brain cancer mortality	National Longitudinal Mortality Study Included individuals with occupational information Included follow-up from 1970-1989 N= 317,968	Cohort study using proportional hazards, Poisson regression techniques, and standardized mortality ratios (SMR)	Interview about current or most recent job within the past 5 years and a job exposure matrix Concentrations: NA	HR (95% CI) For any Pb exposure compared to no exposure: 1.56 (1.00, 2.43) RR (95% CI) from Poisson regression of any Pb exposure compared to no exposure: 1.42 (0.91, 2.20) SMR (95% CI): Not exposed: 0.87 (0.70, 1.06) Any exposure: 1.11 (0.74, 1.59) Note: Effect estimates were greatest among those with high probabilities of exposure and medium/high exposure intensity	Sex, age, race, living in an urban area, marital status and educational level Additional covariate considered but not included: Family income (not used due to large % missing; additional analysis including it gave similar results)

Table 4-49 (Continued): Summary of recent epidemiologic studies of cancer incidence and overall cancer mortality.

Reference (In order of appearance in text)	Study Location	Cancer Outcome	Study Population	Methodological Details	Measure of Pb Exposure/ Concentration Data	Adjusted Effect Estimates	Potential confounders adjusted for in analysis
Rajaraman et al. (2006)	Phoenix, AZ, Boston, MA, and Pittsburgh, PA	Brain cancer incidence	NCI Brain Tumor Study Included individuals ≥18 yr diagnosed with brain cancer less than 8 week before hospitalization; frequency-matched controls were individuals admitted to the same hospitals for non-neoplastic conditions N controls =799 N glioma = 489 N meningioma =197	Case-control study using unconditional logistic regression	Interviews of lifetime work history and exposure databases Concentrations: NA	OR (95% CI) for ever versus no exposure Meningioma: 0.8 (0.5, 1.3) Glioma: 0.8 (0.6, 1.1) Note: positive associations between Pb exposure and meningioma incidence was observed among individuals with ALAD2 genotypes, but not individuals with ALAD1 homozygotes genotypes; these associations were not observed for glioma incidence	Age, sex, race / ethnicity, hospital, and residential proximity to hospital

Table 4-49 (Continued): Summary of recent epidemiologic studies of cancer incidence and overall cancer mortality.

Reference (In order of appearance in text)	**Study Location**	**Cancer Outcome**	**Study Population**	**Methodological Details**	**Measure of Pb Exposure/ Concentration Data**	**Adjusted Effect Estimates**	**Potential confounders adjusted for in analysis**
Bhatti et al. (2009)	Phoenix, AZ, Boston, MA, and Pittsburgh, PA	Brain cancer incidence	NCI Brain Tumor Study Included non-Hispanic whites ≥ 18 yr diagnosed with brain cancer less than 8 week before hospitalization; frequency-matched controls were individuals admitted to the same hospitals for non-neoplastic conditions N controls =494 N glioma = 362 N meningioma =134	Case-control study using unconditional logistic regression	Interviews of lifetime work history and exposure databases Mean (SD) Glioma: 70.5 µg/m^3 per yr (193.8 µg/m^3 per yr) Glioblastoma multiform: 97.5 µg/m^3 per yr (233.9 µg/m^3 per yr) Meningioma: 101.1 µg/m^3 per yr (408.7 µg/m^3 per yr) Controls: 69.7 µg/m^3 per yr (248.8 µg/m^3 per yr)	OR (95% CI) per 100 µg/m^3 per yr increase in cumulative Pb exposure Glioma: 1.0 (0.9, 1.1) Glioblastoma multiform: 1.0 (0.9, 1.1) Meningioma: 1.1 (1.0, 1.2) Note: modification by SNPs was analyzed and associations varied by SNP	Age, sex, hospital, and residential proximity to the hospital

Table 4-49 (Continued): Summary of recent epidemiologic studies of cancer incidence and overall cancer mortality.

Reference (In order of appearance in text)	Study Location	Cancer Outcome	Study Population	Methodological Details	Measure of Pb Exposure/ Concentration Data	Adjusted Effect Estimates	Potential confounders adjusted for in analysis
Breast Cancer:							
Pan et al. (2011)	Canada	Breast cancer incidence	National Enhanced Cancer Surveillance System (NECSS). Population-based sample of cancer cases and controls with information collected from 1994-1997 N controls =2,467 N cases= 2,343	Population-based case-control study using unconditional logistic regression	Self-reported previous addresses and their proximity to Pb smelters (determined using Environmental Quality Database [EQDB]) Concentration: NA	OR (95% CI): Residing >3.2 km from Pb smelter or no nearby smelter: 1.00 Residing 0.8-3.2 km from Pb smelter: 0.41 (0.11, 1.51) Residing <0.8 km from Pb smelter: 0.61 (0.11, 3.42)	Age, province of residence, education, smoking pack years, alcohol consumption, body mass index, recreational physical activity, number of live births, age at menarche, menopausal status, total energy intake, and employment in the industry under consideration Additional covariate considered but not included: Family income

Table 4-49 (Continued): Summary of recent epidemiologic studies of cancer incidence and overall cancer mortality.

Reference (In order of appearance in text)	Study Location	Cancer Outcome	Study Population	Methodological Details	Measure of Pb Exposure/ Concentration Data	Adjusted Effect Estimates	Potential confounders adjusted for in analysis
Other Cancers:							
Rousseau et al. (2007)	Montreal, Canada	Various cancer incidences	Men aged 35-79 yr N population based controls= ranged from 271 to 471 depending on the cancer and exposure of interest N controls with other cancers= ranged from 697 to 2,250 depending on exposure of interest N cancer =ranged from 60 to 442 depending on the cancer and exposure of interest	Population-based case-control study using unconditional logistic regression	Interview of job history and exposure matrix Ever exposed to: Organic Pb 3.0% Inorganic Pb 17.0% Pb in gasoline emissions 38.6%	OR (95% CI): Never exposed is referent group Organic Pb: Esophageal 1.7 (0.5, 6.4) Stomach 3.0 (1.2, 7.3) Colon 1.5 (0.7, 3.6) Rectum 3.0 (1.2, 7.5) Pancreas 0.9 (0.1, 5.2) Prostate 1.9 (0.8, 4.6) Bladder 1.7 (0.7, 4.2) Kidney 2.3 (0.8, 6.7) Non-Hodgkin's lymphoma 0.4 (0.1, 2.2) Note: results are for comparisons using population-based controls; results for controls with other types of cancers were similar except no association was present between organic Pb and rectal cancer	Age, family income, cultural origin or birthplace, and proxy status; all models except those for melanoma and non-Hodgkin's lymphoma were adjusted for smoking

Table 4-49 (Continued): Summary of recent epidemiologic studies of cancer incidence and overall cancer mortality.

Reference (In order of appearance in text)	Study Location	Cancer Outcome	Study Population	Methodological Details	Measure of Pb Exposure/ Concentration Data	Adjusted Effect Estimates	Potential confounders adjusted for in analysis
Rousseau et al. (2007) Continued	Montreal, Canada	Various cancer incidences	Men aged 35-79 yr N population based controls= ranged from 271 to 471 depending on the cancer and exposure of interest N controls with other cancers= ranged from 697 to 2,250 depending on exposure of interest N cancer =ranged from 60 to 442 depending on the cancer and exposure of interest	Population-based case-control study using unconditional logistic regression	Interview of job history and exposure matrix Ever exposed to: Organic Pb 3.0% Inorganic Pb 17.0% Pb in gasoline emissions 38.6%	OR (95% CI): Never exposed is referent group Inorganic Pb: Esophageal 0.6 (0.3, 1.2) Stomach 0.9 (0.6, 1.5) Colon 0.8 (0.5, 1.1) Rectum 0.8 (0.5, 1.3) Pancreas 0.9 (0.4, 1.8) Prostate 1.1 (0.7, 1.6) Bladder 1.1 (0.7, 1.5) Kidney 1.0 (0.6, 1.7) Melanoma 0.4 (0.2, 1.0) Non-Hodgkin's lymphoma 0.7 (0.4, 1.2) Note: results are for comparisons using population-based controls; results for controls with other types of cancers were similar except no association was present between organic Pb and rectal cancer	Age, family income, cultural origin or birthplace, and proxy status; all models except those for melanoma and non-Hodgkin's lymphoma were adjusted for smoking

Table 4-49 (Continued): Summary of recent epidemiologic studies of cancer incidence and overall cancer mortality.

Reference (In order of appearance in text)	Study Location	Cancer Outcome	Study Population	Methodological Details	Measure of Pb Exposure/ Concentration Data	Adjusted Effect Estimates	Potential confounders adjusted for in analysis
Rousseau et al. (2007) Continued	Montreal, Canada	Various cancer incidences	Men aged 35-79 yr N population based controls= ranged from 271 to 471 depending on the cancer and exposure of interest N controls with other cancers= ranged from 697 to 2,250 depending on exposure of interest N cancer =ranged from 60 to 442 depending on the cancer and exposure of interest	Population-based case-control study using unconditional logistic regression	nterview of job history and exposure matrix Ever exposed to: Organic Pb 3.0% Inorganic Pb 17.0% Pb in gasoline emissions 38.6%	OR (95% CI): Never exposed is referent group Pb in gasoline emissions: Esophageal 0.6 (0.4, 1.1) Stomach 1.0 (0.7, 1.4) Colon 0.8 (0.6, 1.1) Rectum 1.0 (0.7, 1.4) Pancreas 0.9 (0.5, 1.4) Prostate 0.9 (0.7, 1.2) Bladder 0.8 (0.6, 1.1) Kidney 1.0 (0.7, 1.5) Melanoma 0.8 (0.5, 1.4) Non-Hodgkin's lymphoma 0.7 (0.5, 1.0) Note: results are for comparisons using population-based controls; results for controls with other types of cancers were similar except no association was present between organic Pb and rectal cancer	Age, family income, cultural origin or birthplace, and proxy status; all models except those for melanoma and non-Hodgkin's lymphoma were adjusted for smoking

Table 4-49 (Continued): Summary of recent epidemiologic studies of cancer incidence and overall cancer mortality.

Reference (In order of appearance in text)	Study Location	Cancer Outcome	Study Population	Methodological Details	Measure of Pb Exposure/ Concentration Data	Adjusted Effect Estimates	Potential confounders adjusted for in analysis
Rousseau et al. (2007) Continued	Montreal, Canada	Various cancer incidences	Men aged 35-79 yr N population based controls= ranged from 271 to 471 depending on the cancer and exposure of interest N controls with other cancers= ranged from 697 to 2,250 depending on exposure of interest N cancer =ranged from 60 to 442 depending on the cancer and exposure of interest	Population-based case-control study using unconditional logistic regression	nterview of job history and exposure matrix Ever exposed to: Organic Pb 3.0% Inorganic Pb 17.0% Pb in gasoline emissions 38.6%	OR (95% CI): Never exposed is referent group Pb in gasoline emissions: Esophageal 0.6 (0.4, 1.1) Stomach 1.0 (0.7, 1.4) Colon 0.8 (0.6, 1.1) Rectum 1.0 (0.7, 1.4) Pancreas 0.9 (0.5, 1.4) Prostate 0.9 (0.7, 1.2) Bladder 0.8 (0.6, 1.1) Kidney 1.0 (0.7, 1.5) Melanoma 0.8 (0.5, 1.4) Non-Hodgkin's lymphoma 0.7 (0.5, 1.0) Note: results are for comparisons using population-based controls; results for controls with other types of cancers were similar except no association was present between organic Pb and rectal cancer	Age, family income, cultural origin or birthplace, and proxy status; all models except those for melanoma and non-Hodgkin's lymphoma were adjusted for smoking

Table 4-49 (Continued): Summary of recent epidemiologic studies of cancer incidence and overall cancer mortality.

Reference (In order of appearance in text)	Study Location	Cancer Outcome	Study Population	Methodological Details	Measure of Pb Exposure/ Concentration Data	Adjusted Effect Estimates	Potential confounders adjusted for in analysis
Santibanez et al. (2008)	Valencia and Alicante, Spain	Esophageal cancer incidence	PANESOES study Included 30-80 yr old men hospitalized in any of the participating study hospitals N controls =285 N cancer =185 (147 squamous cell, 38 adeno. carcinoma)	Case-control study using unconditional logistic regression	Interviews to determine occupational history and a job exposure matrix Concentration: NA	OR (95% CI): All esophageal cancers: Unexposed: 1.00 Low workplace Pb exposure (≤ 4.9 µg/dL): 0.79 (0.43, 1.46) High workplace Pb exposure (>4.9 µg/dL): 1.69 (0.57, 5.03) Esophageal squamous cell carcinoma: Unexposed: 1.00 Low workplace Pb exposure (≤ 4.9 µg/dL): 0.70 (0.34, 1.43) High workplace Pb exposure (>4.9 µg/dL): 0.91 (0.22, 3.75) Adenocarcinoma: Unexposed: 1.00 Low workplace Pb exposure (≤ 4.9 µg/dL): 0.95 (0.32, 2.82) High workplace Pb exposure (>4.9 µg/dL): 5.30 (1.39, 20.22) *Note: associations not changed or slightly increased when restricted to occupational exposures ≥ 15yr	Age, hospital location, educational level, smoking and alcohol use

4.10.1.1 Overall Cancer Mortality

Several recent cohort studies examined the association between Pb concentrations and cancer mortality, including multiple analyses of the NHANES III population. In one NHANES III analysis, the cohort of 13,946 (N for cancer mortality=411) was followed for 12 years and individuals with blood Pb levels greater than 10 µg/dL were excluded from the study (mean baseline blood Pb level was 2.58 µg/dL). No association was observed between blood Pb and cancer mortality (HR of highest tertile [≥ 3.63 µg/dL] compared to lowest tertile [<1.93 µg/dL]: 1.10 [95% CI: 0.82, 1.47]) (Menke et al., 2006). Another analysis of the NHANES III population, which was restricted to individuals 40 years and older at the time of blood Pb collection and included 9,757 (N for cancer mortality=543) individuals with all blood Pb levels (including those greater than 10 µg/dL), reported associations between blood Pb and cancer mortality (Schober et al., 2006). In this study, median follow-up time was 8.6 years. The RRs were 1.69 (95% CI: 1.14, 2.52) for individuals with blood Pb levels of at least 10 µg/dL and 1.44 (95% CI: 1.12, 1.86) for blood Pb levels of 5-9 µg/dL compared to individuals with blood Pb levels less than 5 µg/dL. When stratified by age, point estimates comparing blood Pb levels of 5-9 versus less than 5 µg/dL were similar across all age groups but only statistically significant among 75-84 year olds. The risks of mortality associated with blood Pb levels ≥ 10 µg/dL in the groups aged 40-74 years and 85 years and older were elevated.

A study of men (primarily white) from the greater Boston, MA area enrolled in the Normative Aging Study (NAS) found no association between blood or bone Pb and cancer mortality in adjusted analyses after an average of 8.9 years of follow-up (N=1,038, N for cancer mortality=85 when using blood Pb measures; N=727, N for cancer mortality=57 when using bone Pb measures). At baseline, the mean (SD) blood Pb level for this population was 5.6 (3.4) µg/dL, and blood Pb was poorly correlated with measured bone Pb (Weisskopf et al., 2009). As part of the Study of Osteoporotic Fractures, 533 white women aged 65-87 (N for cancer mortality=38) were included in a sub-study of blood Pb level and cancer mortality and were followed for approximately 12 years (Khalil et al., 2009b). The mean (SD) blood Pb level at baseline was 5.3 (2.3) µg/dL, and no association was detected between blood Pb and cancer mortality in the study population.

Overall, recent epidemiologic studies of blood Pb levels and cancer mortality reported inconsistent results. An epidemiologic study using NHANES III data demonstrated the strongest association between blood Pb and increased cancer mortality; however, other

studies reported weak or no associations. These cohort studies were well-conducted longitudinal studies with control for potential confounders, such as age, smoking, and education (see list of the potential confounders addressed in each study in Table 4-49). One limitation is that the studies in populations other than NHANES cohorts each had a small number of cancer mortality cases.

4.10.1.2 Overall Cancer Incidence

Studies of overall cancer incidence have also been performed (Table 4-49). An ecologic analysis compared Pb-related air pollution over 5 year increments from 1990 to 2005 with incidence rates of cancer during this time period (cancer sites not specified) among children (N not specified) (Absalon and Slesak, 2010). The highest Pb air pollution levels were measured in 1990 when over 50% of the study area exceeded the limit of 1 $\mu g/m^3$ per year. No correlation was observed both overall and in sex-specific analyses. Another study (N=1,940) examined correlations between Pb concentrations in soil, water, vegetation, and hair samples with incidence of neoplasms (Obhodas et al., 2007). The Pb concentrations were not correlated with incidence of neoplasms. A recent study using the 2007-2008 NHANES cohort reported no association between higher creatinine-adjusted urine Pb levels and elevated odds of having ever had cancer or a malignancy (N=1,857) (Mendy et al., 2012). The timing of cancer diagnosis in relation to the urine sample collection was not identified.

Overall, recent epidemiologic studies reported no positive associations between various measures of Pb exposure and overall cancer incidence. These studies are limited by their ecologic and cross-sectional designs. Absalon and Slesak (2010) and Obhodas et al. (2007) did not collect biological measurements, and no control for potential confounding was mentioned.

4.10.1.3 Lung Cancer

Most of the recent evidence regarding lung cancer incidence is provided by a few studies of occupationally-exposed adults. These are described in Table 4-49. Some studies in the 2006 Pb AQCD (U.S. EPA, 2006b) reported associations between Pb exposure and lung cancer in occupational cohorts, although the studies were limited due to possible confounding by smoking or other workplace exposures. In a more recently published study of smelter workers (N=187, N for lung cancer=46), no association was observed between several metrics of Pb exposure (peak blood Pb values, number of years Pb samples were obtained, and cumulative blood Pb index) and lung cancer incidence and

mortality combined (Lundstrom et al., 2006). The median follow-up in the study was about 30 years, and the median peak blood Pb values during employment were 49.7 µg/dL for lung cancer cases and 55.9 µg/dL for controls. In a study of 1,462 tin smelter workers, no association was observed between Pb exposure and lung cancer mortality in unweighted analyses, but when the analyses were weighted by age and time since exposure, positive associations were apparent (Jones et al., 2007). In this study, cumulative Pb exposure was calculated by combining historical air sampling data and personnel record cards, which specified work histories. The median cumulative Pb exposure was estimated to be approximately 2 mg/m^3 per year. It is important to note that the tin smelter workers were exposed to other metals as well, such as As and antimony, and the study did not specify if additional potential confounders were evaluated (beyond the weighting for age and time since exposure). A population-based case-control study performed among men in Montreal, Canada in the 1980s assessed occupational Pb exposure via interviews regarding job histories and determined the likely Pb exposures associated with the job activities (Rousseau et al., 2007). No association was apparent between organic Pb, inorganic Pb, or Pb from gasoline emissions and lung cancer (N ranged from 271 to 1,203 depending on the exposure of interest).

Studies were also conducted that compared lung tissue Pb measurements for individuals with lung cancer to those without lung cancer. The controls for these studies were individuals with metastases in the lung from other primary cancers (De Palma et al., 2008) and individuals with non-cancerous lung diseases (De Palma et al., 2008; Kuo et al., 2006). Limitations in these studies include their cross-sectional design, the measurement of Pb in cancerous tissue, which may have altered Pb distribution, and the use of controls with other cancers and lung diseases. Findings are mixed among the studies. De Palma et al. (2008) reported higher Pb concentrations in the cancerous and non-cancerous lung tissue of individuals with non-small cell lung cancer compared to control groups, although the authors report these results may be confounded by smoking. Kuo et al. (2006) found no statistical difference in Pb levels for lung tissue of individuals with lung cancer compared to controls.

In summary, some studies in the 2006 Pb AQCD reported associations between indicators of Pb exposure and lung cancer among occupational cohorts; however, recent epidemiologic studies of lung cancer reported no associations. Overall, these recent epidemiologic studies included only men, limiting the generalizability. The studies by Jones et al. (2007) and Rousseau et al. (2007) also have the disadvantage of not obtaining actual measures of Pb exposure or biomarker levels. In addition, these studies, as well as those in the 2006 Pb AQCD, are of occupational cohorts, and the relationships with Pb exposures may be confounded by other workplace exposures and covariates that were not considered, such as smoking.

4.10.1.4 Brain Cancer

A few studies of brain cancer examined the association between cancer and occupational Pb exposure using exposures determined via exposure databases and patient interviews about past jobs and known exposures Table 4-49). The National Longitudinal Mortality Study, a study that included a national sample of the U.S. population (N=317,968), estimated occupational Pb exposure based on current/most recent employment among individuals (van Wijngaarden and Dosemeci, 2006). Although not all estimates using various statistical techniques and measures of Pb exposure/intensity are statistically significant, a pattern of increased associations between Pb exposure and brain cancer mortality was observed in the study population. In a case-control study of brain tumors (N for controls=799, N for glioma=489, N for meningioma =197), glioma was reported to have no association with any Pb exposure metric. However, positive associations were observed between high cumulative occupational Pb exposure and meningioma among individuals with *ALAD2* genotypes (OR 2.4 [95% CI: 0.7, 8.8] comparing individuals ever exposed to Pb with those not exposed to Pb; OR 12.8 [95% CI: 1.4, 120.8] comparing individuals with cumulative Pb exposure ≥ 100 μg/m^3 per year to those not exposed to Pb) (Rajaraman et al., 2006). This association was not present among individuals with the *ALAD1* homozygous genotypes (OR 0.5 [95% CI: 0.3, 1.0] comparing individuals ever exposed to Pb with those not exposed to Pb; OR 0.7 [95% CI: 0.2, 1.8] comparing individuals with cumulative Pb exposure ≥ 100 μg/m^3 per year to those not exposed to Pb). Another study of the association between occupational Pb exposure (measured using self-reported occupational exposure history) and brain tumors reported none or slight overall associations with types of brain tumors; however, positive associations were observed among individuals with certain genetic single nucleotide polymorphisms (SNPs) (n for controls=494, n for glioma=362, n for meningioma =134) (Bhatti et al., 2009). After control for multiple comparisons, individuals with the rs1050450 *GPX1* variant had positive associations between cumulative Pb exposure and glioblastoma multiforme and meningioma. Individuals without the rs2239774*RAC2* variant showed a positive association between Pb and glioblastoma multiforme. Also, individuals without the rs7574920 *XDH* variant displayed a positive association between Pb and meningioma.

Overall, associations between occupational Pb exposure and brain cancer incidence and mortality were found to vary according to several genetic variants. Studies of the association between Pb exposure and brain cancer were not reported in the 2006 Pb AQCD. These studies were limited in the implications of their results because they do not have individual level biological or exposure Pb measurements and the potential for confounding by other workplace exposures exists.

4.10.1.5 Breast Cancer

The association between proximity to a Pb smelter and breast cancer was evaluated in a non-occupational cohort. A population-based case-control study in Canada (N for controls=2,467, N for cases=2,343) examined the proximity to a Pb smelter based on residential addresses (Pan et al., 2011) (Table 4-49). No association was reported between proximity of a Pb smelter and breast cancer incidence, but the study was limited by the small number of women who resided near a Pb smelter (n=13 lived ≤ 3.2 km from Pb smelter). No Pb biomarker or exposure data were available.

A few case-control studies examined Pb levels in biological samples among individuals with and without breast tumor and/or cancer. A study of newly diagnosed breast cancer patients and controls examined Pb levels in blood and hair samples and reported higher Pb levels in both for cancer cases, although the difference in the Pb content in hair samples was not statistically significant (Alatise and Schrauzer, 2010). Siddiqui et al. (2006) observed higher blood Pb levels in women with benign and malignant tumors compared to controls. Additionally, although blood Pb levels were higher among those with malignant breast tumors compared to those with benign tumors, both had similar levels of Pb detected in breast tissues. Another study of Pb levels present in breast tissue reported no statistical difference in Pb levels (Pasha et al., 2008b). However, a study of breast tissue did observe a statistically significant difference between Pb levels in the breast tissue of cancer cases and controls (Ionescu et al., 2007). Finally, a study of Pb levels in urine reported a positive association between urine Pb and breast cancer, but this association became null when women taking nonsteroidal aromatase inhibitors but not taking bisphosphonates (a combination responsible for bone loss) were excluded from the analysis (McElroy et al., 2008).

The 2006 Pb AQCD (U.S. EPA, 2006b) did not report any studies examining Pb levels and breast cancer. Overall, recent studies suggest that women with breast cancer may have higher blood Pb levels than those without breast cancer. However, results are mixed in studies that compared breast tissue Pb concentrations between breast tumor and control samples. These studies are limited by their design. The samples are taken after cancer is already present in the cases, thus, the directionality between tissue or blood Pb levels and cancer development cannot be established. Additionally, the sample sizes are often small, and the studies may be underpowered [most of the studies had less than 25 cases (Alatise and Schrauzer, 2010; Ionescu et al., 2007; Siddiqui et al., 2006)]. A case-control study, also limited by its method of exposure measurement, reported no association between living near a Pb smelter and breast cancer (Pan et al., 2011).

4.10.1.6 Other Cancers

There have been a few studies of cancer types other than those listed above. The 2006 Pb AQCD reported evidence of an association between Pb exposure and stomach cancer in several occupational cohorts. A study performed among men in Montreal, Canada in the 1980s evaluated multiple cancer outcomes and estimated occupational exposures to organic Pb, inorganic Pb, and Pb from gasoline emissions via interviews regarding job histories and subsequent exposure approximations by chemists and hygienists (N for cases and controls varied from 60 to 2,250 based on the cancer type and exposure) (Rousseau et al., 2007). Adults with occupational exposure to organic Pb exposure had greater odds of having stomach cancer compared to adults without occupational exposure to organic Pb. A positive association was also observed for rectal cancer when population-based controls were used but was null when the control population was limited to individuals with other types of cancers. No association was detected for cancers of the esophagus, colon, pancreas, prostate, bladder, kidney, melanoma, or non-Hodgkin's lymphoma. None of the cancers were associated with occupational exposure to inorganic Pb. When occupational exposure to Pb in gasoline emissions was categorized as "unexposed," "nonsubstantial level," or "substantial level," a positive association with stomach cancer was observed when cancer controls were used as the comparison group; however, the association was not present when population controls were utilized as the control group). Another case-control study using participant interviews and a job exposure matrix, including only men, (N for controls=285, N for cancer=185) reported no association between occupational Pb exposure and esophageal squamous cell carcinomas, but an association was present between high occupational Pb exposure and adenocarcinoma of the esophagus (Santibanez et al., 2008). However, neither of these studies quantified Pb levels in biological or exposure samples.

Several studies compared Pb levels in blood, tissue, and urine of individuals who have cancer with Pb levels in individuals who are cancer-free. Compared to control groups, higher Pb levels were observed in the blood and bladder tissue of individuals with bladder cancer (Golabek et al., 2009), the kidney tissue of individuals with renal cell carcinoma (with highest levels among those with the highest stage tumors) (Calvo et al., 2009), the tissue (but not serum) of individuals with laryngeal cancer (Olszewski et al., 2006), the blood of individuals with gastric cancer (Khorasani et al., 2008), the plasma and hair of individuals with gastrointestinal cancer (Pasha et al., 2010), the blood and hair of individuals with non-specified types of cancer (Pasha et al., 2008c; Pasha et al., 2007), and the hair of individuals with benign tumors (Pasha et al., 2008a). No statistical difference in Pb levels was reported for colon tissue of individuals with colorectal polyps (Alimonti et al., 2008) or urine of individuals with bladder cancer (Lin et al., 2009) compared to control groups. A study examining Pb levels in kidney tissue reported the

highest levels of Pb in normal kidney tissue samples that were adjacent to neoplastic tumors. The Pb levels reported in the kidney tissue of neoplastic tumors were elevated compared to those detected in corpses without neoplastic tumors of the kidney (Cerulli et al., 2006). All of these comparison studies are limited by the inability to determine temporality as Pb biomarkers were measured after the cancer diagnosis; the level of Pb may be due to changes that result from having cancer, not changes that result in cancer. Many of these studies attempted to control for this by including only cases that have not undergone certain treatments. Additionally, studies are limited by their small sample sizes and the selection of the control populations. Control populations are supposed to represent the general population from which the cases are drawn; some of the control subjects in these studies are individuals with diseases/conditions warranting tissue resections, which are not prevalent in the general population.

In summary, recent epidemiologic studies examining the potential for associations of Pb exposure with the incidence of specific cancers reported varying associations with occupational Pb exposure. Associations were null for occupational Pb exposure and most cancer sites examined. However, a positive association was observed between occupational Pb exposure and adenocarcinoma of the esophagus as well as exposure to occupational organic Pb and stomach cancer, which is supported by evidence of a relationship between Pb exposure and stomach cancer in occupational cohorts reported in the 2006 Pb AQCD. Associations between occupational organic Pb exposure and rectal cancer and occupational exposure to Pb in gasoline emissions and stomach cancer were inconsistent. These studies of various cancer sites have limited generalizability due to the study populations comprising only men. In addition, there are no personal biological or exposure samples used in the epidemiologic analyses and confounding by other occupational exposures is possible. In other studies, biological samples were used in biomarker comparisons of cancer and cancer-free individuals but as stated above, these studies have multiple other limitations.

4.10.1.7 Animal Models of Carcinogenicity

Previous AQCDs have established that Pb has been shown to act as a carcinogen in animal toxicology models, albeit at relatively high concentrations. Chronic oral Pb acetate exposure to male and female rodents has consistently been shown to be a kidney carcinogen in multiple separate studies, inducing adenocarcinomas and adenomas after chronic exposure. Gliomas of the brain have also been reported after oral Pb exposure. The kidneys are the most common target of Pb-induced carcinogenicity (Kasprzak et al., 1985; Koller et al., 1985; Azar et al., 1973; Van Esch and Kroes, 1969) but the testes, brain, adrenals, prostate, pituitary, and mammary gland have also been

affected (IARC, 2006a). The typical cancer bioassays used by IARC or NTP as evidence of Pb-induced carcinogenicity were designed using rodents, typically males but sometimes animals of both sexes, that were continuously exposed to Pb acetate in chow (i.e., 1,000 or 10,000 ppm Pb acetate) or drinking water (i.e., 26 or 2,600 ppm Pb acetate) for 18 months to two years in duration, the typical lifespan of a rodent (Kasprzak et al., 1985; Koller et al., 1985; Azar et al., 1973; Van Esch and Kroes, 1969). These two-year cancer bioassays and the doses employed are typical of cancer bioassays employed for other chemicals, albeit at doses that are higher than Pb doses cited in other toxicological sections of the ISA. In cancer bioassays, to obtain statistically valid data from small groups of animals, doses are selected such that any dose-related effects will occur frequently enough to be detected. The 2006 Pb AQCD (U.S. EPA, 2006b) pointed out that because Pb is a "well-established animal carcinogen…., focus has been more on the mechanism of neoplasia and possible immunomodulatory effects of Pb in the promotion of cancer." This focus continues to date. More recent studies have focused on administration of Pb with known carcinogens or antioxidants such that lifestage, diet, and mode of action can be better understood. Developmental Pb acetate exposure also induced kidney tumors in offspring whose dams received Pb acetate in drinking water during pregnancy and lactation.

Recognition of the importance of windows of exposure in Pb-induced cancer bioassays is a focus of more recent studies. In one study, gestational and lactational exposure of laboratory rodents to inorganic Pb (500, 750 or 1,000 ppm Pb acetate in drinking water) induced carcinogenicity in adult offspring (Waalkes et al., 1995). Another recent study considered Pb-induced carcinogenesis in laboratory animals with early life Pb exposure (gestation, lactation and continued until 8 weeks of age) in which Tokar et al. (2010) examined tumorigenesis in homozygous metallothionein I/II knockout mice and their corresponding wild type controls (groups of ten mice each). The dams/mothers were exposed by drinking water to 2,000 or 4,000 ppm Pb acetate during gestation and lactation and compared to untreated controls. Study animals were exposed in utero, through birth and lactation, and then postnatally to drinking water until 8 weeks old. The Pb-exposed metallothionein I/II knockout mice had increased testicular teratomas and renal and urinary bladder preneoplasia. The tumor burdens of Pb-exposed wild-type mice were not statistically significantly different than controls. The data suggest that metallothionein can protect against Pb-induced tumorigenesis. Concerns with the study are that the doses are at levels of Pb to which humans would not likely be exposed and there is no metallothionein null condition in humans, though there is variability in the expression of metallothionein. The data do not address whether this variability would have any impact on Pb-induced carcinogenesis in humans. Collectively, the animal toxicology data demonstrate that Pb is a well-established animal carcinogen in studies employing high-dose Pb exposure over a continuous extended duration of exposure

(i.e., 2 years), which is typical of cancer bioassays. Newer studies are showing early-life maternal Pb exposure can contribute to carcinogenicity in offspring and suggest that metallothionein is protective against cancer in this pathway.

4.10.2 Cancer Biomarkers

Studies of Pb exposure and prostate specific antigen (PSA), a biomarker for prostate cancer, were not reported in the 2006 Pb AQCD (U.S. EPA, 2006b). A recent cross-sectional study of men aged 21-40 years without occupational history of exposure to metals examined PSA (N=57). This study reported a positive association between Pb levels and PSA levels (measured in the same blood samples) in regression models adjusted for the following potential confounders: age, smoking, alcohol consumption, and other metals (Cd, Zn, Se, and Cu) (Pizent et al., 2009). The median concurrent blood Pb level was 2.6 µg/dL (range 1.0-10.8 µg/dL). The authors note that the study population was young and at lower risk of prostate cancer than are older men.

4.10.3 Modes of Action for Pb-induced Carcinogenicity

Most evidence clearly supports the mode of action of Pb inducing carcinogenicity in animal models, but the exact chain of events supporting this segue has not been completely characterized. Specifically, it is unclear whether the mode of action of Pb is best understood within the framework of multistage carcinogenesis, genomic instability or epigenetic modification. For example, multistage carcinogenesis involves a series of cellular and molecular changes that result from the progressive accumulation of mutations that induce alterations in cancer-related genes. Pb does not appear to follow this paradigm, and the literature suggests it is weakly mutagenic. Pb does appear to have some ability to induce DNA damage (Section 4.10.3.2). However, the ability of Pb to alter gene expression through epigenetic mechanism (Section 4.10.3.3) and to interact with proteins may be a means by which Pb induces carcinogenicity. It is known that Pb can replace Zn in Zn-binding (Zn-finger) proteins (Section 4.2), which include hormone receptors, cell-cycle regulatory proteins, the Ah receptor, estrogen receptor, p53, DNA repair proteins, protamines, and histones. These Zn-finger proteins all bind to specific recognition elements in DNA. Thus, Pb may act at a post-translational stage to alter protein structure of Zn-finger proteins, which can in turn alter gene expression, DNA repair and other cellular functions. To recapitulate, cancer develops from one or a combination of multiple mechanisms including modification of DNA via epigenetics or enzyme dysfunction and genetic instability or mutation. These modifications then provide

the cancer cells with a selective growth advantage. In this schematic, Pb may contribute to epigenetic changes and chromosomal aberrations.

The genomic instability paradigm requires a cascade of genome-wide changes caused by impaired DNA repair, kinetochore assembly, cellular checkpoints, centrosome duplication, microtubule dynamics or a number of cell maintenance processes. These processes have been rarely studied for Pb, thus there are few data that suggest Pb may interfere with some of these processes. Furthermore, the bulk of the literature in this area involves Pb chromate and it is unclear if the effects are due to Pb or chromate. Epigenetic modifications may lead to cancer by altering cellular functions without altering the DNA sequence. The most commonly studied epigenetic change is methylation alterations. A small number of studies show that Pb can induce epigenetic changes (Section 4.10.3.3), but studies are still missing to clearly tie these effects to Pb-induced carcinogenesis and genotoxicity. Thus, either genomic instability or epigenetic modification paradigms or some combination of the two may underlie Pb-induced carcinogenicity.

Exposure to mixtures can also contribute to understanding of modes of action. No recent studies of the protective role of Ca^{2+} or Zn in Pb-induced carcinogenesis or genotoxicity were found. Pb can displace these and other divalent cations, affecting physiological processes. There were some data suggesting that metallothionein (Section 4.10.4), which sequesters Pb and makes it less bioavailable, protects rodents from Pb-induced cancers. Boron, melatonin, N-acetylcysteine, turmeric and myrrh protected cells against Pb-induced genotoxicity (Section 4.10.3.2) and affected antioxidant status, especially the glutathione pathway. There were some data suggesting that Pb mimics or antagonizes the essential micronutrient Se in rodents. These data are discussed in more detail below (Section 4.10.4) and point to the relevance of mixtures in assessing toxicity.

4.10.3.1 Neoplastic Transformation Studies, Human Cell Cultures

Carcinogenesis can be measured in cell culture systems through neoplastic transformation models that monitor change by following morphological transformation of cells, i.e., formation of a focus (or foci) of cell growth. Xie et al. (2007) treated BEP2D cells (human papilloma virus-immortalized human bronchial cells) with 0, 1, 5, or 10 µg/cm^2 Pb chromate for 120 hours. $PbCrO_4$ induced foci formation in a concentration-dependent manner. Xie et al. (2008) treated BJhTERT cells (hTERT-immortalized human skin fibroblasts) and ATLD-2 cells (hTERT-immortalized human skin fibroblasts deficient in Mre11) with 0, 0.1, 0.5, and 1 µg/cm^2 Pb chromate for 120 hours. $PbCrO_4$ induced foci formation in a concentration-dependent manner in the Mre11 deficient cells. Mre11 is a component of the MRN complex and plays a role in telomere maintenance and double-

strand break repair. Mre11 was required to prevent Pb chromate-induced neoplastic transformation.

Immune Modulation of Tumorigenesis by Pb

As described in the 2006 Pb AQCD (U.S. EPA, 2006b), Pb-induced immunotoxicity can contribute to increased risk of cancer, primarily as the result of suppressed Th1 responses and misregulated inflammation. First, Pb-induced misregulation of inflammation involving innate immune cells has been shown to result in chronic insult to tissues. These insults, excessive lipid and DNA oxidation production by overproduction of ROS and weakened antioxidant defenses, can increase the likelihood of mutagenesis, cellular instability, and tumor cell formation. For example, results from Xu et al. (2008) support the association with Pb exposure and DNA damage, and investigators concluded that it is a possible route to increased Pb-induced tumorigenesis. The second component of increased risk of cancer involves Pb-induced suppression of Th1-dependent anti-tumor immunity as acquired immunity shifts statistically significantly toward Th2 responses. With cytotoxic T lymphocytes and other cell-mediated defenses dramatically lessened, the capacity to resist cancer may be compromised.

4.10.3.2 DNA and Cellular Damage

Multiple studies have been performed examining the relationship between Pb and DNA and cellular damage. Details of the recent epidemiologic and toxicological studies follow.

Epidemiologic Evidence for DNA and Cellular Damage

Multiple studies examined the relationship between blood Pb and sister chromatid exchange (SCE). SCEs are exchanges of homologous DNA material between chromatids on a chromosome and are a test for mutagenicity or DNA damage. A study of male policemen reported a mean blood Pb level for the study population of 43.5 µg/dL (Wiwanitkit et al., 2008). In analyses dichotomized as high or low blood Pb levels (cut-off at 49.7 µg/dL), the higher blood Pb group was observed to have higher mean SCE. Another study of adult males compared the SCE of storage battery manufacturing workers (mean blood Pb level of 40.14 µg/dL) and office workers (mean blood Pb level of 9.77 µg/dL) (Duydu et al., 2005). The exposed workers had higher SCE levels and also a greater number of cells in which the SCEs per cell were higher than the 95th percentile of the population. Finally, a study of children aged 5-14 years old (mean [SD] blood Pb level of 7.69 [4.29] µg/dL) reported no correlation between blood Pb levels and SCE

(Mielzyńska et al., 2006). However, the study did report a positive association between blood Pb and micronuclei (MN) levels.

Other studies of DNA damage have reported mixed results. A study of children ages 6-11 years old and environmentally-exposed to Pb reported no association between blood Pb and baseline DNA damage or repair ability after a peroxide challenge (children attending a school far from a Pb smelter: median blood Pb level 4.6 µg/dL; children attending a school near a Pb smelter: median blood Pb level 28.6 µg/dL) (Méndez-Gómez et al., 2008). Another study included adult participants aged 50-65 years and reported an association between blood Pb and carcinoembryonic antigen (CEA) but not with DNA-strand breaks, MN frequency, or oxidative DNA damage (median blood Pb level of the study population: 3.92 µg/dL) (De Coster et al., 2008). A study conducted among workers exposed to Pb (mean blood Pb level: 30.3 µg/dL) and unexposed controls (mean blood Pb level: 3.2 µg/dL) reported greater cytogenetic damage (measured by MN frequency), chromosomal aberrations, and DNA damage in the Pb-exposed group (although this was not statistically significant in linear regression models controlling for age) (Grover et al., 2010). A study of painters in India, where Pb concentrations in paint are high, reported a mean (SD) blood Pb level of 21.56 (6.43) µg/dL among painters who reported painting houses for 8-9 hours/day for 5-10 years (Khan et al., 2010b); the mean (SD) blood Pb level was 2.84 (0.96) µg/dL for healthy workers who had not been occupationally exposed to Pb. Cytogenetic damage was higher among the painters compared to the healthy controls. Another study compared the blood Pb of metal workers and office workers and reported higher blood Pb levels (both current and 2 year average) among the metal workers (blood Pb level $\geq$ 20 µg/dL) compared to the office workers (blood Pb level <10 µg/dL) (Olewińska et al., 2010). Overall, the workers had increased DNA strand breaks versus the office workers (this held true at various blood Pb levels). Finally, a study of Pb battery workers with symptoms of Pb toxicity and a group of controls were examined (Shaik and Jamil, 2009). Higher chromosomal aberrations, MN frequency, and DNA damage were reported for the battery workers as compared to the controls. These workplace studies are limited by the lack of consideration for potential confounding factors, including other occupational exposures.

Toxicological Evidence for DNA and Cellular Damage

Sister Chromatid Exchanges

Pb has been shown to induce SCEs both in vivo and in vitro. Tapisso et al. (2009), considered SCEs in adult Algerian mice (groups of six mice each) that were treated by i.p. injection with 5 or 10 doses of 0.46 mg/kg Pb acetate. The SCE in bone marrow were

elevated after Pb exposure alone and increased with time. Co-exposure with Cd or Zn further increased SCE levels.

SCE was also followed in cultured human cells. Ustundag and Duydu (2007) considered the ability of N-acetylcysteine and melatonin to reduce Pb nitrate-induced SCE in a single human donor. Cells were treated with 0, 1, 5, 10, or 50 µM Pb nitrate. SCE statistically significantly increased at every Pb concentration in a concentration dependent manner. Both 1 and 2 mM N-acetylcysteine and melatonin were able to statistically significantly reduce SCE levels in Pb-exposed cells. In another study, Turkez et al. (2011) considered the ability of boron compounds, essential micronutrients, to prevent Pb chloride-induced SCE in human lymphocytes. Cells were obtained from 4 non-smoking donors. Both 3 and 5 ppm Pb chloride induced a statistically significant increase in SCE levels over controls. Boron was able to statistically significantly diminish these levels. For both studies, exposure times were not provided, and the full interpretation of these data is limited by the limited number of donors and the absence of an exposure time for the SCE assay.

Micronuclei Formation

The 2006 Pb AQCD stated "studies of genotoxicity consistently find associations of Pb exposure with DNA damage and MN formation" and recent studies continue to report these associations. Alghazal et al. (2008b) considered the ability of Pb acetate trihydrate to induce MN in bone marrow of adult Wistar rats. Animals were given a daily dose of 100 mg/L in their drinking water for 125 days. The mean number of MN in male and female rats was statistically significantly higher in Pb-exposed animals than in unexposed controls. Tapisso et al. (2009) considered Pb-induced MN in rodents. Algerian mice were treated by i.p. injection with 5 or 10 doses of 0.46 mg/kg Pb acetate and compared to untreated controls. The MN in bone marrow were elevated after Pb exposure and increased with time.

MN formation has also been followed in cultured human cells. Ustundag and Duydu (2007) considered the ability of N-acetylcysteine and melatonin to reduce Pb nitrate-induced MN in a single human donor. Cells were treated with 0, 1, 5, 10, or 50 µM Pb nitrate. MN formation statistically significantly increased at the two highest Pb concentrations in a concentration-dependent manner. Both 1 and 2 mM N-acetylcysteine and melatonin were not able to statistically significantly reduce MN levels. In another study, Turkez et al. considered the ability of boron compounds to prevent Pb chloride-induced MN in human lymphocytes. Cells were obtained from 4 non-smoking donors. Both 3 and 5 ppm Pb chloride induced a statistically significant increase in MN levels over controls. Boron induced a statistically significant attenuation of these Pb-induced levels. For both studies, exposure times were not provided, and the full interpretation of

these data is limited by the limited number of donors and the absence of an exposure time for the MN assay. Gastaldo et al. (2007) evaluated the ability of Pb to induce MN. Human endothelial HMEC cell line was treated with 1–1,000 µM Pb nitrate for 24 hours. MN increased in a statistically significant, concentration-dependent manner.

Hypoxanthine-guanine phosphoribosyltransferase Mutations

The potential mutagenicity of Pb in human or animal cells has been evaluated by monitoring mutations at the hypoxanthine-guanine phosphoribosytransferase (HPRT) locus. Li et al. (2008a) evaluated Pb acetate-induced HPRT in the non-small-cell lung carcinoma tumor cell line, CL3, and in normal human diploid fibroblasts (specific tissue source not reported). All cells were exposed to 0, 100, 300, or 500 µM Pb acetate for 24 hours in serum-free medium ± a 1-hour pretreatment with a MKK1/2 inhibitor or a PKC-alpha inhibitor. Pb alone did not induce HPRT mutations. Inhibiting the ERK pathway via either inhibitor statistically significantly increased Pb-induced mutagenesis. Wang et al. (2008c), investigated Pb acetate-induced HPRT mutations in CL3 cells. All cells were exposed to 0, 100, 300 or 500 µM Pb acetate for 24 hours in serum-free medium ± a 1-hour pretreatment with a PKC-alpha inhibitor or siRNA for PKC-alpha. Pb alone did not induce HPRT mutations. Inhibiting PKC-alpha via either inhibitor statistically significantly increased Pb-induced mutagenesis. McNeill et al. (2007) examined Pb acetate-induced HPRT mutations in Chinese hamster ovary AA8 cells and AA8 cells overexpressing human Ape1. Cells were treated with 5 µM Pb acetate for 6 hours. No increases in HPRT mutations were observed after Pb exposure in either cell line but with specific pathway perturbations (PKC-alpha or ERK), Pb was able to induce HPRT mutations.

Chromosomal Aberrations

Chromosomal aberrations, another indicator of cancer risk, were followed in Pb-exposed rodents (El-Ashmawy et al., 2006). Dietary exposure to Pb acetate administered as a single dose of 5,000 ppm w/w to adult male Swiss albino mice caused statistically significant increased levels of chromosomal aberrations in the Pb treatment alone group, particularly with respect to fragments, deletions, ring chromosomes, gaps, and end-to-end associations. In addition, the authors found turmeric and myrrh powders were protective against the effects of Pb. Concerns with the study include the use of only a single dose of Pb acetate along with the high levels of unusual aberrations such as ring chromosomes and end-to-end associations. Typically, these aberrations are rare after metal exposure, but were the most commonly observed aberration in this study raising questions about the quality of the metaphase preparations. An additional concern was that only 50 metaphases per dose were analyzed instead of the more common 100 metaphases per

dose. The authors did not explain why their spectrum of aberrations was so different, why they only used one dose, or analyzed fewer metaphases per dose.

Multiple studies considered the ability of Pb to induce chromosomal aberrations in cultured human cells. The ability of Pb nitrate to induce chromosomal aberrations was examined in primary human peripheral blood lymphocytes obtained from healthy, nonsmoking donors (Pasha Shaik et al., 2006). Cells were treated with 0, 1,200 or 2,000 µM Pb nitrate for 2 hours. No increase in chromosomal aberrations was reported. Some aneuploidy was observed. Concerns with the study are that only a 2-hour exposure was used, which may not be long enough for DNA damage to be expressed as a chromosomal aberration. It also appears from the data presentation that only three subjects were used; one for a control, one for the low dose and one for the high dose. Experiments were not repeated, thus given the small number of subjects, this study may not have had sufficient power to detect any effects. Holmes et al. (2006a), treated WHTBF-6 cells (hTERT-immortalized human lung cells) with 0, 0.1, 0.5, or 1 µg/cm^2 Pb chromate for 24-120 hours or with 0, 0.1, 0.5, 1, 5 or 10 µg/cm^2 Pb oxide for 24 or 120 hours. Pb chromate induced statistically significant, concentration-dependent increases in centrosome abnormalities and aneuploidy; lead oxide did not induce significant changes. Wise et al. (2006a) treated BEP2D cells with 0, 0.5, 1, 5, or 10 µg/cm^2 Pb chromate for 24 hours. Pb chromate induced statistically significant concentration-dependent increases in chromosomal aberrations. Holmes et al. (2006b), treated WHTBF-6 cells with 0, 0.1, 0.5, or 1 µg/cm^2 Pb chromate for 24-72 hours. Pb chromate induced statistically significant, concentration-dependent increases in chromosomal aberrations. The effects of the chromate anion cannot be ruled out as causative in inducing these chromosomal aberrations. Wise et al. (2006b), treated WHTBF-6 cells with 0, 0.1, 0.5, or 1 µg/cm^2 Pb chromate for 24-120 hours. Pb chromate induced statistically significant, concentration-dependent increases in spindle assembly and checkpoint disruption, effects of mitosis and aneuploidy. By contrast, chromate-free Pb oxide did not induce centrosome amplification. The effects were likely attributable to the chromate anion. Xie et al. (2007) treated BEP2D cells with 0, 1, 5, or 10 µg/cm^2 Pb chromate for 24 hours. Pb chromate induced statistically significant, concentration-dependent increases in chromosomal aberrations and aneuploidy. Wise et al. (2010) treated WHTBF-6 cells with 0, 0.1, 0.5, or 1 µg/cm^2 Pb chromate for 24 hours in a study comparing 4 chromate compounds. Pb chromate induced statistically significant, concentration-dependent increases in chromosomal aberrations.

Multiple investigators considered the ability of Pb chromate to induce chromosome aberrations in rodent cell cultures. Grlickova-Duzevik et al. (2006) treated Chinese hamster ovary (CHO) cells with 0, 0.1, 0.5, or 1 µg/cm^2 Pb chromate for 24 hours. Specific CHO lines used included AA8 (wildtype) EM9 (XRCC1-deficient), and H9T3

(EM9 complemented with human XRCC1 gene). Pb chromate induced statistically significant, concentration-dependent increases in chromosomal aberrations that were statistically significantly increased by deficiency of the DNA repair gene *XRCC1*. Nestmann and Zhang (2007) treated Chinese hamster ovary cells (clone WB(L)) with 0, 0.1, 0.5, 1, 5, or 10 µg/cm^2 Pb chromate (as pigment yellow) for 18 hours. No increases in chromosomal aberrations were observed. Savery et al. (2007) treated CHO cells with 0, 0.1, 0.5, 1, or 5 µg/cm^2 Pb chromate for 24 hours. Specific CHO lines used included AA8 (wildtype), KO40 (*Fancg*-deficient), and 40BP6 (*Fancg*-complemented). The *Fancg* gene plays an important role in cellular resistance to DNA interstrand crosslinks, protecting against genetic instability. Pb chromate induced statistically significant, concentration-dependent increases in chromosomal aberrations that were increased by *Fancg*-deficiency. Camyre et al. (2007) treated CHO cells with 0, 0.1, 0.5, 1, 5, or 10 µg/cm^2 Pb chromate for 24 hours. Specific CHO lines used included CHO-K1 (parental), xrs-6 (Ku80 deficient), and 2E (xrs-6 complemented with Ku80 gene). Pb chromate induced statistically significant, concentration-dependent increases in chromosomal aberrations that were not affected by Ku80 deficiency. Ku80 is a gene involved in nonhomologous end-joining repair and its absence can contribute to genetic instability. Stackpole et al. (2007) treated CHO and Chinese hamster lung (CHL) cells with 0, 0.1, 0.5, or 1 µg/cm^2 Pb chromate for 24 hours. Specific CHO lines used included AA8 (wildtype), irs1SF (XRCC3-deficient), and 1SFwt8 (XRCC3 complemented). XRCC3 is DNA repair enzyme involved in homologous recombination. CHL lines used included V79 (wildtype), irs3 (Rad51C deficient) and irs3#6 (Rad51C complemented). Rad51C is a gene that encodes strand-transfer proteins that are thought to be involved in recombinational repair of damaged DNA and in meiotic recombination. Pb chromate induced statistically significant, concentration-dependent increases in chromosomal aberrations that were statistically significantly increased by both XRCC3 and Rad51C deficiency.

Multiple studies considered the ability of Pb chromate to induce chromosome aberrations in marine mammal cell cultures. Li Chen et al. (2009) treated primary North Atlantic right whale lung and skin fibroblasts with 0, 0.5, 1.0, 2.0, and 4.0 µg/cm^2 Pb chromate for 24 hours. Wise et al. (2009) treated primary Steller sea lion lung fibroblasts with 0, 0.1, 0.5, 1 and 5 µg/cm^2 Pb chromate for 24 hours. Wise et al. (2011) treated primary sperm whale skin fibroblasts with 0, 0.5, 1, 3, 5, and 10 µg/cm^2 Pb chromate for 24 hours. In all three studies, Pb chromate induced statistically significant, concentration-dependent increases in chromosomal aberrations.

In summary, exposure of various cell models and an in vivo model to Pb (acetate, chromate, or nitrate) induced significant increases in chromosomal aberration that often responded in a concentration-dependent manner. The use of various cell lines deficient in

specific DNA repair enzymes helped to elucidate which pathways may be most sensitive to Pb-dependent chromosomal aberration. However, a number of studies used Pb chromate exposures and the effects of the chromate anion cannot be ruled out as causative in inducing these chromosomal aberrations.

COMET Assay

Multiple studies considered the ability of Pb to induce DNA single strand breaks in laboratory animals and human and animal cells using the COMET assays. The COMET assay measures DNA damage assessed by single cell electrophoresis of a lysed cell and measurement of the fragmented DNA or tail length. Xu et al. (2008) examined DNA damage in male ICR mice treated with Pb acetate. Animals (5 per group) were given Pb acetate by gavage at doses of 0, 10, 50, or 100 mg/kg body weight every other day for 4 weeks. Pb exposure statistically significantly increased both tail length and tail moment in a concentration-dependent manner. Nava-Hernandez et al. (2009) considered the ability of Pb acetate to induce DNA damage in primary spermatocyte DNA of male Wistar rats. Animals (3 per group) were treated for 13 weeks with 0, 250, or 500 mg/L Pb in their drinking water. There was statistically significantly less DNA damage in the controls compared to the two treatment groups. Narayana and Al-Bader (2011) examined DNA damage in liver tissue of adult male Wistar rats exposed to Pb nitrate. Animals (8 per group) were treated for 60 days with doses of 0, 5,000, or 10,000 ppm Pb nitrate in their drinking water. There were no statistical differences between treated groups and controls. Drosophila melanogaster larvae (72 hours old) exposed to Pb nitrate (2,000, 4,000, and 8,000 µM in culture media for 24 hours) yielded haemocytes that tested positive in the COMET assay; Pb chloride (8,000 µM) did not cause DNA damage with the COMET assay (Carmona et al., 2011).

Other studies used the COMET assay in cultured human cells. Pasha Shaik et al. (2006) treated primary human peripheral blood lymphocytes obtained from healthy, nonsmoking donors with 0, 2.1×, 2.4×, 2.7×, 3.0×, and 3.3×10^3 µM Pb nitrate for 2 hours and found concentration-dependent increases in COMET tail length. Concerns with the study are that apparently no negative control was used. It also appears from the data presentation that only five subjects were used; one for each dose. Experiments were not repeated. Thus, given the small number of subjects and the absence of a negative control, this study may only be detecting background levels of DNA damage. Xie et al. (2008) treated BJhTERT cells (hTERT-immortalized human skin fibroblasts) and ATLD-2 cells (hTERT-immortalized human skin fibroblasts deficient in Mre11) with 0, 0.1, 0.5, and 1 µg/cm_2 Pb chromate for 24 hours. Pb chromate induced a concentration-dependent increase in DNA double strand breaks measured by the COMET assay. Pb chromate exposure and the effects of the chromate anion cannot be ruled out as causative in

inducing these aberrations. In another study, Pb nitrate exposure (30 µg/mL) induced statistically significant increased DNA damage in human liver HepG2 cells that was attenuated with co-exposure with the antioxidant NAC (500 µM) (Yedjou et al., 2010).

Other studies used the COMET assay to examine Pb-induced DNA single strand breaks in rodent cell cultures. Xu et al. (2006), treated PC12 cells with 0, 0.1, 1 or 10 µM Pb acetate. Both tail length and tail moment statistically significantly increased in a concentration-dependent manner. Kermani et al. (2008) exposed mouse bone marrow-mesenchymal stem cells to 60 µM Pb acetate for 48 hours. There was an increase in several COMET assay measurements including tail length.

The COMET assay showed multiple positive findings of DNA damage after Pb exposure in rodents, flies, primary human cells, and cell lines. In vivo studies with rodents exposed to Pb acetate yielded significant increases in tail length and moment via COMET assays in separate studies that used lymphocytes and sperm. In *Drosophila*, Pb nitrate but not Pb chloride produced significant increases with the COMET assay. Human cell culture from primary cells (lymphocytes) and from cell lines (fibroblasts and liver) had increased DNA damage as assessed using the COMET assays with separate Pb nitrate and Pb chromate exposures. Thus, the COMET assay showed multiple positive findings of DNA damage after in vitro and in vivo Pb exposure.

Other Indicators of DNA Damage

Other studies considered the ability of Pb to induce DNA double strand breaks by measuring gamma-H2A.X foci formation in cultured human cells. Xie et al. (2008) treated BJhTERT cells (hTERT-immortalized human skin fibroblasts) and ATLD-2 cells (hTERT-immortalized human skin fibroblasts deficient in Mre11) with 0, 0.1, 0.5, and 1 µg/cm^2 Pb chromate for 24 hours. Pb chromate induced a concentration-dependent increase in DNA double strand breaks measured by gamma-H2A.X foci formation. Pb chromate exposure and the effects of the chromate anion cannot be ruled out as contributory. Gastaldo et al. (2007) evaluated the ability of Pb to induce DNA double strand breaks with both gamma-H2A.X foci formation and pulse-field gel electrophoresis in cultured human cells. The human endothelial HMEC cell line was treated with 1 to 1,000 µM Pb nitrate for 24 hours. DNA double strand breaks increased in a concentration-dependent manner. Wise et al. (2010) treated WHTBF-6 cells with 0, 0.1, 0.5, or 1 µg/cm^2 Pb chromate for 24 hours in a study comparing four chromate compounds. Pb chromate induced statistically significant, concentration-dependent increases in DNA double strand breaks measured by gamma-H2A.X foci formation, at a similar level to the three other compounds. A few studies demonstrated the ability of Pb to destabilize DNA by forming DNA-histone cross links, which can lead to histone aggregation (Rabbani-Chadegani et al., 2011; Rabbani-Chadegani et al., 2009). In

extracts of rat liver, Pb nitrate (<300 µM) was shown to react with chromatin components and induce chromatin aggregation via histone-DNA cross links.

Genotoxicity testing of *Drosophila melanogaster* larvae (72 hours old) using the Wing Spot test showed that neither Pb chloride nor Pb nitrate (at concentrations of 2,000, 4,000 and 8,000 µM in culture media with exposure until pupation) was able to induce significant increases in the frequency of wing spots (Carmona et al., 2011). The wing spot test can detect mitotic recombination and multiple mutational events such as point mutations, deletions, and certain types of chromosome aberrations (Graf and Würgler, 1986). Further, wing spot assays employing Pb co-exposure with gamma radiation showed no effect of Pb on gamma radiation induced spotting frequency.

Multiple studies examined the effects of Pb on DNA repair. Most were conducted in cultured cells, and one was done in an animal model. El-Ghor et al. (2011) followed microsatellite instability (MSI) in Pb acetate trihydrate exposed adult male rats. MSI reflects impaired DNA mismatch repair and contributes to an increased risk of cancer. DNA from leukocytes of adult male albino rats exposed to Pb acetate (acute: single oral dose of 467 mg/kg BW or sub-chronic: 47 mg/kg BW six days/week for 4 week) showed increased MSI at three microsatellite loci (D6mit3, D9mit2, and D15Mgh1). This study is limited by its small sample size (n=2 to 3 rodents per treatment group). Li et al. (2008a) evaluated Pb acetate-induced effects on nucleotide excision repair efficiency in CL3 cells. All cells were exposed to 0, 100, 300 or 500 µM Pb acetate for 24 hours in serum-free medium. Pb increased nucleotide excision repair efficiency. Gastaldo et al. (2007) evaluated the ability of Pb to affect DNA repair in cultured human cells. The human endothelial HMEC cell line was treated with 100 µM Pb nitrate for 24 hours. Pb inhibited non-homologous end joining repair, over activated MRE11-dependent repair, and increased Rad51-related repair. Xie et al. (2008) treated BJhTERT cells (hTERT-immortalized human skin fibroblasts) and ATLD-2 cells (hTERT-immortalized human skin fibroblasts deficient in Mre11) with 0, 0.1, 0.5, and 1 µg/cm^2 Pb chromate for 24 or 120 hours. Mre11 was required to prevent Pb chromate-induced DNA double strand breaks. In this finding, Pb chromate exposure and the effects of the chromate anion cannot be ruled out as causative. McNeill et al, (2007) considered Pb acetate effects on Ape1. Chinese hamster ovary cells (AA8 subtype) were treated with 0, 0.5, 5, 50, or 500 µM Pb acetate and then whole cell extracts were used to determine AP site incision activity. The data show that Pb reduced AP endonuclease function. Finally, studies considered Pb-induced cellular proliferation in laboratory animals. Kermani et al. (2008) exposed mouse bone marrow-mesenchymal stem cells to 0-100 µM Pb acetate for 48 hours. As measured by the MTT assay, Pb decreased cell proliferation at all concentrations tested. An earlier study in rats showed Pb nitrate-induced increased proliferation of liver cells after a partial hepatectomy, with more prominent effects found

in males than females (sexual dimorphism) (Tessitore et al., 1995). Recent studies showed similar trends in males. Fortoul et al. (2005) exposed adult male CD1 mice (24 animals per group) to 1×10^4 µM Pb acetate, 0.006 M Cd chloride or a mixture of the two chemicals for 1 hour twice a week for 4 weeks by inhalation. Electron microscopy indicated Pb-induced cellular proliferation in the lungs.

4.10.3.3 Epigenetics

Air pollution exposure is being linked increasingly with epigenetic changes in humans and toxicological models (Pavanello et al., 2010; Baccarelli and Bollati, 2009; Tarantini et al., 2009; Bollati et al., 2007). Epigenetic changes are changes in gene expression that occur without actual changes in the DNA sequence, and these changes may be heritable. Epigenetic changes are mediated by histone modification, DNA methylation, miRNA changes, or pathways that affect these three mediators. Differential epigenetic modification has the possibility to contribute to disease. Epigenetic studies have been conducted to examine the associations between Pb biomarker levels and global DNA methylation markers [Alu and long interspersed nuclear element-1 (LINE-1)] in humans (Wright et al., 2010; Pilsner et al., 2009). Wright et al. (Wright, 2013; Wright et al., 2010) examined men from the Normative Aging Study (N=517) with mean (SD) Pb levels of 20.5 (14.8) µg/g in tibia, 27.4 (19.7) µg/g in patella, and 4.1 (2.4) µg/dL in blood. In both crude and adjusted analyses, patella Pb levels were inversely associated with LINE-1 methylation but not with Alu. The adjusted models all included age, body mass index, percent lymphocytes, with some adjusted models also controlling for education, smoking, and blood Pb levels. In examination of the relationship between patella Pb and LINE-1 more closely, a non-linear trend was observed with a smaller magnitude of effect estimated for higher patella Pb (≥ 40 µg/g). No associations were observed for tibia or blood Pb and either LINE-1 or Alu. Another study included maternal-infant pairs from the Early Life Exposures in Mexico to Environmental Toxicants study (N=103) and measured LINE-1 and Alu methylation in umbilical cord blood samples (Pilsner et al., 2009). In unadjusted models, maternal tibia Pb levels one month postpartum (mean [SD]: 10.5 [8.4] µg/g) were inversely associated with Alu methylation in the cord blood. Maternal patella Pb levels one month postpartum (mean [SD]: 12.9 [14.3] µg/g) were inversely associated with LINE-1 methylation. The associations persisted in models adjusted for maternal age, maternal education, sex of infant, smoking during pregnancy, and umbilical cord blood Pb levels (the results were no longer statistically significant when umbilical cord blood was removed from the model). No association was detected between umbilical cord Pb levels and the DNA methylation markers. Overall, the studies consistently demonstrate an association

between higher patella Pb levels and lower LINE-1 methylation. Lower DNA methylation is associated with increased gene expression; however, the link between global DNA methylation and risk of disease, has not been established.

Toxicological studies have examined Pb-induced epigenetic changes and gene expression, DNA repair, and mitogenesis. Glahn et al., (2008) performed a gene array study in primary normal human bronchial epithelial cells from four donors after in vitro treatment of the cells with 55 µg/dL Pb chloride, 15 µg/L Cd sulfate, 25 µg/L Co chloride or all three combined for 72 hours. The authors describe a pattern of RNA expression changes indicating "…coordinated stress-response and cell-survival signaling, deregulation of cell proliferation, increased steroid metabolism, and increased expression of xenobiotic metabolizing enzymes." These are all known targets of possible epigenetic changes, but attributing the results to epigenetic changes is complicated. In a recent publication (Li et al., 2011), exposure of HepG2 cells to a high dose of Pb (100 µM Pb acetate) resulted in ALAD gene promoter hypermethylation and decreased ALAD transcription. This was in agreement with findings in battery plant workers who showed ALAD hypermethylation (versus non-occupationally exposed controls) and an association of this hypermethylation with elevated risk of Pb poisoning (Li et al., 2011). These latter results have implications for Pb toxicokinetics or disposition of Pb as modified by ALAD.

4.10.4 Effects of Pb within Mixtures

Several studies considered the impact of Pb as part of a mixture on genotoxicity and mutagenesis. Mendez-Gomez et al. (2008) evaluated 65 children in Mexico with high environmental exposures to both As and Pb. DNA damage and decreased DNA repair were seen with increased proximity to a Pb smelter using the COMET assay and other assays but did not correlate with blood Pb levels and no interaction between blood Pb and urinary As levels was observed. Tapisso et al. (2009) examined Pb alone, Pb plus Zn and Pb plus Cd-induced MN in rodents. Algerian mice (groups of six mice each) were treated i.p. with 5 or 10 doses of 0.46 mg/kg Pb acetate and compared to untreated controls. The MN in bone marrow were elevated after Pb treatment alone and increased with time. Co-exposure with Cd or Zn did not further increase MN levels but did increase SCE levels. Glahn et al. (2008) performed a gene array study in primary normal human bronchial epithelial cells from four donors treated with 55 µg/dL Pb chloride, 15 µg/L Cd sulfate, 25 µg/L Co chloride or all three combined for 72 hours. There was a clear interaction of all three metals impacting RNA expression.

Studies in the 2006 Pb AQCD (U.S. EPA, 2006b) found a protective role for calcium in genotoxic and mutagenic assays with Pb co-exposure. No recent studies of the protective role of calcium in Pb-induced carcinogenesis or genotoxicity were found. There were some data suggesting that boron, melatonin, N-acetylcysteine, turmeric and myrrh protect cells against Pb-induced genotoxicity (Section 4.10.3.2).

A recent study details Pb and Se interactions in virus-dependent carcinogenesis in laboratory animals. Schrauzer (2008) considered the impact of Se on carcinogenesis by studying four groups of weanling virgin female C3H/St mice infected with murine mammary tumor virus (groups of 20-30 mice), which induces mammary tumor formation. One set of two groups were fed a diet containing 0.15 ppm Se and then were exposed via drinking water to acetic acid (control group) or 0.5 ppm Pb acetate. The second set of two groups were fed a diet containing 0.65 ppm Se and then similarly exposed to acetic acid or 0.5 ppm Pb acetate. The study was primarily focused on the general effects of a low Se diet. The data suggest that Se is anticarcinogenic as in the groups without Pb exposure, the animals exposed to the higher Se levels had fewer mammary tumors and these tumors had a delayed onset of appearance. Pb exposure with low Se caused the same delayed onset as did the higher dose of Se and also caused some reduction in the tumor frequency. Pb exposure with higher Se increased the tumor frequency and the onset of the tumors. Pb also induced weight loss at 14 months in both exposed groups. The data suggest that there may be interactions of Pb and Se, but they suggest that Pb mimics or antagonizes Se. They do not suggest that Se is protective of Pb-induced toxicity or carcinogenesis.

In summary, the new data on Pb exposure as part of a mixture are derived from studies designed with co-exposure to metals or antioxidants. Children in Mexico living near a Pb smelter with co-exposure to high levels of Pb and As showed elevated DNA damage and impaired DNA repair but no conclusions were able to be made regarding whether this was a result of exposure to individual metals or a combination of metals. Pb and Cd co-exposure in mice elevated SCE levels but did not further exacerbate MN levels above Pb exposure alone. Primary lung cells exposed to a metals mixture showed an interaction at the mRNA level among the three metals tested. In other genotoxicity assays, various antioxidants (melatonin, NAC, turmeric and myrrh) and metals (boron) were protective against Pb-induced genotoxicity. In an animal model of breast cancer, Se modified the onset and multiplicity of murine mammary tumor virus-induced tumorogenicity in Pb-exposed animals. These data show that co-exposure of Pb with antioxidants or metals, modifies the effect of Pb on DNA damage, DNA repair, mutagenicity, genotoxicity, or tumorogenicity.

4.10.5 Summary and Causal Determination

Toxicological and epidemiologic studies of the association between Pb exposure and cancer and cancer-related outcomes have been reviewed in the preceding sections. Evaluation of the relationship between Pb exposure and cancer with respect to causality was based primarily on evidence for tumor incidence in experimental animals and evidence describing potential modes of action including mutagenesis, DNA aberrations, and epigenetic changes. Supporting evidence was provided from associations of Pb exposure with cancer incidence and mortality in humans. The application of the key supporting evidence from these studies to the causal framework is summarized in Table 4-50 and the following text.

The toxicological literature provides consistent evidence of the carcinogenic potential of Pb and possible contributing modes of action, including genotoxic, mutagenic and epigenetic effects. In laboratory studies, chronic Pb exposure for 18 months or two years to high concentrations such as 10,000 ppm Pb acetate in diet or 2,600 ppm Pb acetate in drinking water has been demonstrated to be an animal carcinogen. Chronic Pb exposure to male and female rodents has consistently induced kidney and brain carcinogenesis in multiple separate studies, inducing various tumors, (i.e., adenocarcinomas, adenomas, and gliomas. Pb has also been shown to cause mammary gland, prostate, adrenal, and testicular tumors in animals. Developmental Pb acetate exposure also induced tumors in offspring whose dams received Pb acetate in drinking water during pregnancy and lactation. Multiple toxicological studies showed neoplastic transformation in cultured cells providing an additional potential mode of action, but most used Pb chromate, and it is possible that the chromate ion contributed to these findings. The toxicological and epidemiologic literature provides evidence for potential carcinogenic modes of action from genotoxic, mutagenic and epigenetic assays.

Multiple longitudinal epidemiologic studies have been performed examining the association between cancer incidence and mortality and Pb exposures, estimated with biological measures and exposure databases. Mixed results have been reported for cancer mortality studies; a large NHANES epidemiologic study demonstrated a positive association between baseline blood Pb and cancer mortality with median 8.6 years of follow up on subjects (Schober et al., 2006), but the other studies reported null results (Khalil et al., 2009b; Weisskopf et al., 2009; Menke et al., 2006). These were well-conducted epidemiologic studies with control for important potential confounders such as age, smoking, and education. Although the 2006 Pb AQCD (U.S. EPA, 2006b) reported some studies that found an association between Pb exposure indicators and lung cancer, recent studies mostly included occupationally-exposed adults and observed no associations. Most studies of Pb and brain cancer were null among the overall study

population, but positive associations were observed among individuals with certain genetic variants. However, the studies of Pb and brain cancer were all limited due to possible confounding by several factors, including other workplace exposures. A limited amount of research has been performed on other types of cancer. The 2006 Pb AQCD reported evidence that suggested an association between Pb exposure and stomach cancer, but in a recent study of stomach cancer the results were inconsistent, reporting a positive association between organic Pb exposure and stomach cancer but null findings for exposure to inorganic Pb or Pb from gasoline emissions and stomach cancer.

Among epidemiologic studies, high Pb levels (over 40 µg/dL in adults) were associated with SCEs among adults. This association was not observed among children (mean concurrent blood Pb 7.69 µg/dL). Other epidemiologic studies of DNA damage reported inconsistent results. Consistent with previous toxicological findings, Pb does appear to have genotoxic activity in animal and in vitro models, inducing SCE, MN and DNA strand breaks. The majority of the chromosomal aberration studies with Pb-induced significant findings used Pb chromate exposure and the aberrations are likely due to the chromate. Pb does not appear to be strongly mutagenic as the HPRT assays were typically negative unless a cell signaling pathway was disturbed.

Mechanistic understanding of the carcinogenicity of Pb in toxicological models is expanding with work on the antioxidant Se and metallothionein, a protein that binds Pb and reduces its bioavailability. Low Se diet affects tumorigenesis and tumor multiplicity with Pb exposure. Metallothionein has been shown to be protective against the effect of Pb on carcinogenicity. Pb is clastogenic and mutagenic in some but not all models. Clastogenicity and mutagenicity may be possible mechanisms contributing to cancer but are not absolutely associated with the induction of cancer. Because Pb has a higher atomic weight than does Zn, Pb replaces Zn at many Zn binding sites or Zn finger proteins. This substitution has the potential to induce effects that can indirectly contribute to carcinogenicity via interactions with hormone receptors, cell-cycle regulatory proteins, tumor suppressor genes like p53, DNA repair enzymes, histones, etc. These indirect effects may act at a post-translational level to negatively alter protein structure and DNA repair.

Epigenetic changes associated with Pb exposure or biological markers of Pb exposure are beginning to appear in the literature. Epigenetic modifications may contribute to carcinogenicity by altering DNA repair or changing the expression of a tumor suppressor gene or oncogene. A small number of epidemiologic studies examining Pb and global epigenetic changes demonstrated an inverse association between Pb concentrations and LINE-1 or Alu methylation. Lower DNA methylation is associated with increased gene expression, but epigenetic contributions to cancer are not yet fully characterized in this

emerging area of research. Toxicological studies show that Pb can activate or interfere with a number of signaling and repair pathways, though it is unclear whether these are due to epigenetic responses or genotoxicity. Thus, an underlying mechanism is still uncertain, but likely involves genomic instability, epigenetic modifications, or both.

In conclusion, the toxicological literature provides the strong evidence for long-term exposure (i.e., 18 months or 2 years) to high concentrations of Pb (> 2,600 ppm) and cancer. The consistent evidence indicating Pb-induced carcinogenicity in animal models is substantiated by the mode of action findings from multiple high-quality toxicological studies in animal and in vitro models from different laboratories. This is substantiated by the findings of other agencies including IARC, which has classified inorganic Pb compounds as a probable human carcinogen and the National Toxicology Program, which has listed Pb and Pb compounds as "reasonably anticipated to be human carcinogens." Strong evidence from animal toxicological studies demonstrates an association between Pb and cancer, genotoxicity or epigenetic modification. Carcinogenicity in animal toxicology studies with relevant routes of Pb exposure has been reported in the kidneys, testes, brain, adrenals, prostate, pituitary, and mammary gland, albeit at high doses of Pb. Based on such evidence, IARC has classified inorganic Pb compounds as a probable human carcinogen and the National Toxicology Program (NTP) has listed Pb and Pb compounds as "reasonably anticipated to be human carcinogens." Epidemiologic studies of cancer incidence and mortality reported inconsistent results; one strong epidemiologic study demonstrated an association between blood Pb and increased cancer mortality, but the other studies reported weak or no associations. In the 2006 Pb AQCD, indicators of Pb exposure were found to be associated with stomach cancer, and a recent study on stomach cancer and occupational Pb exposure reported mixed findings depending on the type of Pb exposure (organic Pb, inorganic Pb, or Pb from gasoline emissions). Similarly, some studies in the 2006 Pb AQCD reported associations between Pb exposure indicators and lung cancer. Recent epidemiologic studies of lung cancer focused on occupational exposures and reported inconsistent associations. The majority of epidemiologic studies of brain cancer had null results overall, but positive associations between Pb exposure indicators and brain cancer were observed among individuals with certain genotypes. Overall, the consistent and strong body of evidence from toxicological studies on tumor incidence and potential modes of action but inconsistent epidemiologic evidence is sufficient to conclude that a causal relationship is likely to exist between Pb exposure and cancer.

Table 4-50 Summary of evidence supporting cancer and genotoxicity causal determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Exposure or Blood Levels Associated with Effects
Cancer - Likely Causal			
Consistent evidence from multiple animal studies with chronic Pb exposure	Consistent findings across multiple toxicology studies using 18 month or two year cancer bioassays in rats wherein rodents are fed chow or received drinking water enriched with Pb acetate and show tumor development.	Azar et al. (1973), Kasprzak et al. (1985), Koller et al. (1985), Van Esch and Kroes (1969)	Chronic 10,000 ppm Pb acetate diet or 2,600 ppm drinking water Pb acetate, no blood Pb measurement available.
	Gestational and lactational Pb exposure induced carcinogenicity in adult offspring.	Waalkes et al., (1995) Section 4.10.1.7	500, 750 and 1,000 ppm Pb in drinking water, no blood Pb measurement available.
Most evidence clearly supports mode of action	Consistent toxicological evidence for mutagenicity, carcinogenicity, and genotoxicity of Pb reported by multiple laboratories in humans, animals and in vitro models using multiple assays (MN, SCE, COMET).	Epidemiology evidence of DNA and cellular damage: See Section 4.10.3.2 Toxicology evidence of DNA and cellular damage: See Section 4.10.3.2	
	Some evidence for epigenetic changes. Bone Pb levels were inversely associated with LINE-1 methylation in a study of adult men.		
	Study showed inverse association between maternal postpartum bone Pb levels and Alu and LINE-1 methylation in cord blood.	See Section 4.10.3.3	
	Occupational battery workers had ALAD hypermethylation compared with controls; cell culture study of high dose Pb exposure caused ALAD hypermethylation.		
Toxicological evidence of clastogenic, mutagenic, and genotoxic effects with Pb chromate	Some toxicological studies employ Pb chromate when investigating the clastogenic, mutagenic, and genotoxic effects of Pb. The effect of the chromate ion in contributing to these effects cannot be ruled out.	See Section 4.10.3.2	

Table 4-50 (Continued): Summary of evidence supporting cancer and genotoxicity causal determinations.

Attribute in Causal Framework[a]	Key Evidence[b]	References[b]	Pb Exposure or Blood Levels Associated with Effects
Epidemiologic evidence is limited and inconsistent	Epidemiologic studies of overall cancer mortality have inconsistent findings. These are high-quality, longitudinal studies with an average follow-up of at least 8.6 years and control for potential confounders, such as age, smoking, and education. There is uncertainty related to exposure patterns resulting in likely higher past Pb exposure. Epidemiologic studies of specific cancer sites were limited. Recent studies were not consistent with previous findings of possible associations for lung and stomach cancers reported in the 2006 Pb AQCD. Many of the epidemiologic studies examining specific cancer sites were case-control studies and not all included potentially important confounders, such as smoking. There is uncertainty related to exposure patterns resulting in likely higher past Pb exposure.	Menke et al. (2006) Schober et al. (2006) Weisskopf et al. (2009), Khalil (2009b) Overall Cancer Mortality: See Section 4.10.1.1 Specific Cancer: See Sections: (4.10.1.3, Lung); (4.10.1.4, Brain); (4.10.1.5 Breast); (4.10.1.6 Other cancers).	In the mortality studies, the majority of study participants' baseline blood Pb levels were <10 µg/dL (those that reported means ranged from 2.58 to 5.6 µg/dL). In studies of specific cancer types, exposure measurements varied (e.g. report of ever exposure, work history, blood Pb levels).

[a]Described in detail in Table II of the Preamble.

[b]Describes the key evidence and references contributing most heavily to causal determination and where applicable to uncertainties or inconsistencies. References to earlier sections indicate where full body of evidence is described.

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CHAPTER 5 POTENTIALLY AT-RISK POPULATIONS

Introduction

The NAAQS are intended to protect public health with an adequate margin of safety. In so doing, protection is provided for both the population as a whole and those groups potentially at increased risk for health effects from exposure to the air pollutant for which each NAAQS is set (Preface to this ISA). To facilitate the identification of populations at increased risk for Pb-related health effects, studies have evaluated various factors that may contribute to susceptibility and/or vulnerability to Pb. The definitions of susceptibility and vulnerability vary across studies, but in most instances "susceptibility" refers to biological or intrinsic factors (e.g., age, sex) while "vulnerability" refers to nonbiological or extrinsic factors (e.g., socioeconomic status [SES]) (U.S. EPA, 2010, 2009a). Additionally, in some cases, the terms "at-risk" and "sensitive" populations have been used to encompass these concepts more generally. In this ISA, "at-risk" groups are defined as those with characteristics that increase the risk of Pb-related health effects in a population. These characteristics include various factors, such as genetic background, race and ethnicity, sex, age, diet, pre-existing disease, SES, and characteristics that may modify exposure or the response to Pb.

Individuals, and ultimately populations, could experience increased risk for air pollutant induced health effects via multiple avenues. A group with intrinsically increased risk would have one or more factors that increase risk for an effect through a biological mechanism. In general, people in this category would have a steeper concentration-risk relationship or would manifest effects at a lower exposure, compared to those not in the category. Potential factors that are often considered intrinsic include genetic background and sex. A group of people could also have extrinsically increased risk, which would be through an external, non-biological factor. Examples of extrinsic factors include SES and diet. Some groups are at risk of increased internal dose at a given exposure concentration. In addition, some groups could have greater exposure (concentration x time), regardless of the delivered dose. Finally, there are those who might be placed at increased risk for experiencing a greater exposure by being exposed at a higher concentration. An example of this is people living near Pb smelters.

Some factors described above are multifaceted and may influence the risk of an air pollutant related health effect through a combination of avenues. For example, SES may

affect access to medical care, which itself may contribute to the presence of preexisting diseases and conditions considered as intrinsic factors. Additionally, children's outdoor activities can lead to more hand-to-mouth contact with contaminated soil than adults, which leads to increased intake dose and exposure. At the same time, children have biological (i.e., intrinsic) differences from adults that may influence their uptake, metabolism, storage, and excretion.

The emphasis of this chapter is to identify and understand the factors that potentially increase the risk of Pb-related health effects, regardless of whether the increased risk is due to intrinsic factors, extrinsic factors, increased dose/exposure, or a combination due to the often interconnectedness of factors. The following sections examine factors that potentially lead to increased risk of Pb-related health effects and characterize the overall weight of evidence for each factor.

Approach to Classifying Potential At-Risk Factors

To identify factors that potentially lead to some populations being at greater risk of Pb-related health effects, the evidence across relevant scientific disciplines (i.e., exposure sciences, dosimetry, toxicology, and epidemiology) was evaluated. In this systematic approach, the collective evidence is used to examine coherence of effects across disciplines and determine biological plausibility. The collective results across the scientific disciplines comprise the overall weight of evidence that is used to determine whether a specific factor results in a population being at increased risk of an air pollutant related health effect. The first section of this chapter (Section 5.1) summarizes physiological factors that influence Pb levels in the body. The second section of this chapter (Section 5.2) summarizes information on factors potentially related to differential Pb exposure. The studies presented in this section supplement the material provided in Chapter 2 and Chapter 3 by examining how factors such as age, sex, race and ethnicity, SES, proximity to Pb sources, and residential factors may affect Pb exposure or blood Pb levels. The third section of this chapter (Section 5.3) discusses the epidemiologic and toxicological studies evaluated in Chapter 4 that provide information on factors potentially related to increased risk of Pb-induced health effects through the use of analysis targeting specific factors. To examine whether Pb differentially affects certain populations, the epidemiologic studies discussed in Section 5.3 conduct stratified analyses to identify the presence or absence of effect measure modification. A thorough evaluation of potential effect measure modifiers may help identify populations that are at increased risk for Pb-related health effects. Highlighted studies include only those where the population was stratified into subgroups (e.g., males versus females or smokers versus nonsmokers) for comparative analysis. In the case of many biomarker studies and

the epidemiologic studies considered, this approach allowed for a comparison between populations exposed to similar Pb concentrations and within the same study design. Toxicological studies also provide evidence of Pb effects and biological plausibility for factors that may lead to increased risk for Pb-related health effects. Included toxicological studies discussed in Section 5.3 may have categorized the study populations by different factors, such as age, sex, diet/nutrition status, and genetics, or are those that examined animal models of disease. These epidemiologic and toxicological studies provide the scientific basis for an overall weight of the evidence evaluation for the identification of specific populations potentially at risk of Pb-related health effects. Details on the magnitude of effects for studies in this third section (Section 5.3) are included in summaries of the studies presented in Chapter 4.

Numerous studies that focused on only one potentially at-risk population were described in previous chapters (Chapter 4) but are not discussed in detail in this chapter because they lacked stratified analysis with adequate comparison groups. For example, pregnancy is a lifestage with potentially increased risk for mothers and fetuses, but because there are no comparison groups for stratified analyses, these studies were presented in Chapter 4 but are not included here. Additionally, it is understood that some of the stratified variables/factors discussed in this third section (Section 5.3) may not be effect measure modifiers but instead may be mediators of Pb-related health effects. Mediators are factors that fall on the causal pathway between Pb exposure and health outcomes, whereas effect measure modifiers are factors that result in changes in the measured associations between Pb exposure and health effects. Because mediators are caused by Pb exposure and are also intermediates in the disease pathway that is studied, mediators are not correctly termed “at-risk” factors. Some of the factors discussed in this third section could be mediators and/or modifiers. These are noted in Table 5-5.

Building on the causal framework discussed in detail in the Preamble and used throughout the ISA, conclusions are made regarding the strength of evidence for each factor that may contribute to increased risk of a Pb-related health effect based on the evaluation and synthesis of evidence across scientific disciplines. The conclusions drawn considered the “Aspects to Aid in Judging Causality” discussed in Table I of the Preamble. The categories considered for evaluating the potential increased risk of an air pollutant-related health effect are “adequate evidence,” “suggestive evidence,” “inadequate evidence,” and “evidence of no effect.” They are described in more detail in Table 5-1.

Table 5-1 Classification of evidence for potential at-risk factors.

	Health Effects
Adequate evidence	There is substantial, consistent evidence within a discipline to conclude that a factor results in a population or lifestage being at increased risk of air pollutant-related health effect(s) relative to some reference population or lifestage. Where applicable this includes coherence across disciplines. Evidence includes multiple high-quality studies.
Suggestive evidence	The collective evidence suggests that a factor results in a population or lifestage being at increased risk of an air pollutant-related health effect relative to some reference population or lifestage, but the evidence is limited due to some inconsistency within a discipline or, where applicable, a lack of coherence across disciplines.
Inadequate evidence	The collective evidence is inadequate to determine if a factor results in a population or lifestage being at increased risk of an air pollutant-related health effect relative to some reference population or lifestage. The available studies are of insufficient quantity, quality, consistency, and/or statistical power to permit a conclusion to be drawn.
Evidence of no effect	There is substantial, consistent evidence within a discipline to conclude that a factor does not result in a population or lifestage being at increased risk of air pollutant-related health effect(s) relative to some reference population or lifestage. Where applicable this includes coherence across disciplines. Evidence includes multiple high-quality studies.

5.1 Physiological Factors that Influence the Internal Distribution of Pb

Blood and bone Pb measures are influenced to varying degrees by biokinetic processes (e.g., absorption, distribution, metabolism, excretion), which are discussed in detail in Chapter 3. These processes can be affected by multiple factors, such as age, genetics, diet, and co-exposure with other metals and non-metals.

Age influences the biokinetic response to Pb within the body. Infants may be considered an at-risk population because Pb easily crosses the placental barrier and accumulates in fetal tissue during gestation (Pillai et al., 2009; Wang et al., 2009e; Uzbekov et al., 2007). This transfer of Pb from mother to fetus is partly due to the remobilization of the mother's bone stores (O'Flaherty, 1998; Franklin et al., 1997). This also results in increased maternal blood Pb levels (Lamadrid-Figueroa et al., 2006; Gulson et al., 2004a; Hertz-Picciotto et al., 2000; Gulson et al., 1997; Lagerkvist et al., 1996; Schuhmacher et al., 1996; Rothenberg et al., 1994a). Infants also absorb Pb more efficiently from their gastrointestinal tracks than older children and adults (Mushak, 1991; Ziegler et al., 1978). Bone growth rate is high during childhood. The majority of a child's Pb body burden is not permanently incorporated in the bone, but some Pb does remain in the bone until older age (McNeill et al., 2000; O'Flaherty, 1995; Leggett, 1993). Older adults are more

likely to have age-related degeneration of bones and organ systems and a possible redistribution of Pb stored in the bones into the blood stream (Popovic et al., 2005; Garrido Latorre et al., 2003; Gulson et al., 2002).

Various genes can also affect Pb biomarker concentrations. Genetic variants of the vitamin D receptor (VDR) in humans have been associated with varied bone and plasma Pb levels (Rezende et al., 2008; Theppeang et al., 2004; Schwartz et al., 2000a). Multiple studies have also examined the association between the aminolevulinate dehydratase (ALAD) polymorphism and blood Pb levels and found that the ALAD-2 polymorphism may be biologically related to varying Pb levels, although some studies report no difference for ALAD alleles (see also Section 4.2.3) (Miyaki et al., 2009; Shaik and Jamil, 2009; Sobin et al., 2009; Chen et al., 2008c; Rabstein et al., 2008; Scinicariello et al., 2007; Zhao et al., 2007; Montenegro et al., 2006; Wananukul et al., 2006).

It is well established that diets sufficient in minerals such as calcium (Ca), iron (Fe), and zinc (Zn) offer some protection from Pb exposure by preventing or competing with Pb for absorption in the GI tract. A study in China reported that children who regularly consumed breakfast had lower blood Pb levels than those children that did not eat breakfast after controlling for confounders such as urban-rural location of the school, maternal education level and occupation, and paternal occupation (Liu et al., 2011a). Diets designed to limit or reduce caloric intake and induce weight loss have been associated with increased blood Pb levels in adult rats (Han et al., 1999). A toxicological study reported negative effects of Pb on osmotic fragility, TBARS production, catalase activity, and other oxidative parameters, but most of these effects were reduced to the levels observed in the control group when the rats were given supplementation of zinc and vitamins (Massó-González and Antonio-García, 2009). Toxicological studies by Jamieson et al. (2008; 2006) also reported that a zinc-deficient diet increases bone and renal Pb content and impairs skeletal growth and mineralization in rats. A zinc-supplemented diet attenuated bone and renal Pb content. Toxicological studies among rats and chicks have shown that dietary deficiency of calcium induces increased Pb absorption and retention (Mykkanen and Wasserman, 1981; Six and Goyer, 1970). Increased calcium intake reduces accumulation of Pb in bone and mobilization of Pb during pregnancy and lactation in rats (Bogden et al., 1995). Additionally, human studies have reported that Fe deficiencies may result in higher Pb absorption or altered biokinetics (Schell et al., 2004; Marcus and Schwartz, 1987; Mahaffey and Annest, 1986).

In summary, age, genetics, and diet affect the biokinetics of Pb, which in turn affects the internal distribution of Pb. These factors were discussed in greater detail in Chapter 3

where more information on overall biokinetic and physiological factors affecting Pb distribution is provided.

5.2 Population Characteristics Potentially Related to Differential Pb Exposure

Elevated or differential Pb exposure and related biomarker levels (such as blood Pb), have been shown to be statistically related to several population characteristics, including age, sex, race and ethnicity, SES, proximity to Pb sources, and residential factors (U.S. EPA, 2006b). In most cases, exposure, absorption, and biokinetics of Pb are all influenced to varying degrees by such characteristics. Additionally, the relative importance of such population characteristics in affecting exposure, absorption, and biokinetics varies among individuals in a population and is difficult to quantify. This section presents recent studies demonstrating a relationship between each population characteristic and exposure status. The studies presented in this section build upon the current body of literature suggesting that population characteristics differentially influence Pb exposure; the new literature does not alter previous understanding of the differential influence of population characteristics on Pb exposure. Differential response to given Pb exposures is discussed in Section 5.3.

5.2.1 Age

5.2.1.1 Early Childhood

Typically, children have higher exposure to Pb compared with adults because children's behaviors and activities include hand-to-mouth contact, crawling, and poor hand-washing that typically result in greater Pb ingestion compared with adults (U.S. EPA, 2006b). Children can also have increased Pb exposure because outdoor activities can lead to hand-to-mouth contact with contaminated soil. For example, Zahran et al. (2010) observed that a 1% reduction in soil Pb concentration led to a 1.55 µg/dL reduction in median blood Pb levels ($p < 0.05$) among New Orleans children.

Age of the children may influence blood Pb levels through a combination of behavioral and biokinetic factors. The 2009-2010 NHANES data are presented in Table 5-2 by age and sex. Among children, highest blood Pb levels occurred in the 1-5 year age group (children under age 1 were not included), and within this subgroup (not shown on the table), 1-year old children had the highest blood Pb levels (99th percentile: 9.47 µg/dL)

(CDC, 2011b) (CDC, 2013). It is possible that high blood Pb levels among these young children may also be related to in utero exposures resulting from maternal Pb remobilization from bone stores from historic exposures (Miranda et al., 2010) or from contemporaneous Pb exposures if the mothers had appreciable current Pb exposure. Jones et al. (2009a) analyzed the NHANES datasets for the years 1999-2004 to study trends in blood Pb among two different age groups of children over time (see Table 5-3). They observed greater percentages of children aged 1-2 years having blood Pb levels of 2.5 to <5 µg/dL, 5 to <7.5 µg/dL, and ≥ 10 µg/dL, compared with 3-5 year-old children, but no age difference was noted for the 7.5 to <10 µg/dL bracket. At the same time, 1-2 year-old children had lower percentages of blood Pb levels <1 µg/dL and 1 to <2.5 µg/dL compared with children ages 3-5 years old. This implies that there is a shift in the distribution of blood Pb levels as children age, even during early childhood. These distribution differences may be attributable to differences in exposure (including behavioral influences, such as hand-to-mouth contact and crawling in proximity to contaminated surfaces), residual contributions from the mother's Pb burden, age-dependent variability in biokinetics or diet (e.g., milk versus solid diets). Yapici et al. (2006) studied the relationship between blood Pb level and age among a cohort of children between 6 and 73 months of age with elevated blood Pb levels (87.6% of study group with blood Pb greater than 20 µg/dL) living near a Turkish coal mine. They observed a low but statistically significant negative correlation between blood Pb and age ($r = -0.38$, $p < 0.001$).

Table 5-2 Blood Pb levels (µg/dL) by age and sex, 2009-2010 NHANES.

Age	Sex	N	Avg.	Std. Dev.	5%	25%	50%	75%	95%	99%
1-5 yr	Total	836	1.61	1.49	0.53	0.85	1.21	1.81	4.00	8.03
	Male	429	1.59	1.32	0.51	0.83	1.22	1.84	4.09	7.49
	Female	407	1.64	1.65	0.54	0.90	1.20	1.77	3.69	9.59
6-11 yr	Total	1009	1.05	0.74	0.42	0.61	0.83	1.22	2.36	4.29
	Male	521	1.10	0.73	0.45	0.66	0.88	1.30	2.37	4.18
	Female	488	0.99	0.75	0.38	0.58	0.79	1.12	2.35	3.98
12-19 yr	Total	1183	0.84	0.68	0.33	0.50	0.69	0.96	1.82	3.10
	Male	632	0.98	0.69	0.40	0.58	0.80	1.11	2.09	3.91
	Female	551	0.69	0.62	0.30	0.44	0.57	0.79	1.31	2.25
20-59 yr	Total	3856	1.50	1.83	0.44	0.72	1.08	1.70	3.53	7.27
	Male	1843	1.88	2.33	0.56	0.92	1.37	2.12	4.49	9.68
	Female	2013	1.15	1.10	0.40	0.61	0.89	1.35	2.63	4.41
60+ yr	Total	1909	2.09	1.51	0.72	1.16	1.69	2.53	4.79	8.28
	Male	941	2.46	1.78	0.87	1.39	1.99	2.90	5.56	9.89
	Female	968	1.73	1.07	0.65	1.01	1.43	2.14	3.75	5.42
Overall	Total	8793	1.50	1.57	0.43	0.72	1.10	1.76	3.66	7.21
	Male	4366	1.75	1.88	0.50	0.84	1.29	2.05	4.31	8.62
	Female	4427	1.25	1.13	0.39	0.63	0.96	1.48	2.97	5.17

Source: (CDC, 2013).

Table 5-3 Percentage of children within six categories/brackets of blood Pb levels, 1999-2004 NHANES.

	N	Geometric mean[a]	Percentage (%) of children within categories/brackets (95% CIs)					
			<1 µg/dL	1 to <2.5 µg/dL	2.5 to <5 µg/dL	5 to <7.5 µg/dL	7.5 to <10 µg/dL	≥ 10 µg/dL
Overall	2,532	1.9 (1.8-2.0)	14.0 (11.6-16.6)	55.0 (52.1-57.9)	23.6 (21.1-26.1)	4.5 (3.3-5.9)	1.5 (1.0-2.1)	1.4 (1.0-2.0)
Sex								
Female	1,211	1.9 (1.7-2.0)	14.1 (10.8-17.7)	54.5 (51.1-57.8)	23.9 (20.3-27.8)	4.5 (3.3-5.8)	1.4 (0.8-2.3)	1.7 (0.9-2.6)
Male	1,321	1.9 (1.7-2.0)	14.0 (11.4-16.7)	55.5 (51.4-59.5)	23.2 (20.3-26.3)	4.6 (3.0-6.5)	1.5 (0.9-2.3)	1.3 (0.7-2.6)
Age								
1-2 yr	1,231	2.1 (2.0-2.2)	10.6 (7.7-13.9)	51.0 (46.7-55.3)	27.9 (24.9-31.0)	6.7 (5.0-8.6)	1.4 (0.8-2.2)	2.4 (1.4-3.5)
3-5 yr	1,301	1.7 (1.6-1.9)	16.2 (12.9-19.9)	57.6 (53.8-61.4)	20.7 (17.9-23.7)	3.1 (1.9-4.6)	1.5 (0.8-2.3)	0.9 (0.4-1.5)
Race/Ethnicity								
Non-Hispanic Black	755	2.8 (2.5-3.0)	4.0 (2.5-5.7)	42.5 (37.8-47.2)	36.2 (33.1-39.3)	9.4 (6.9-12.2)	4.6 (3.0-6.5)	3.4 (1.8-5.5)
Mexican American	812	1.9 (1.7-2.0)	10.9 (8.6-13.4)	61.0 (56.9-65.1)	22.1 (18.0-26.5)	3.4 (2.2-5.0)	1.3 (0.6-2.2)	1.2 (0.4-2.6)
Non-Hispanic White	731	1.7 (1.6-1.8)	17.6 (14.0-21.5)	57.1 (52.4-61.7)	19.7 (16.1-23.5)	3.6 (1.9-5.8)	0.8 (0.3-1.6)	1.2 (0.6-2.0)
Poverty-Income Ratio (PIR)								
≤ 1.3	1,302	2.4 (2.2-2.5)	6.7 (4.6-9.2)	49.3 (44.9-53.7)	32.5 (28.6-36.4)	6.9 (2.2-8.8)	2.8 (1.7-4.1)	1.8 (1.1-2.7)
>1.3	1,070	1.5 (1.4-1.6)	19.9 (16.3-23.8)	60.4 (56.9-63.8)	16.0 (12.9-19.3)	2.3 (1.2-3.7)	0.6 (0.1-1.4)	0.8 (0.3-1.6)

[a]Geometric mean Pb Units: µg/dL (95% CI)

Source: Reprinted with permission of the American Academy of Pediatrics; Jones et al. (2009a)

Fetal and child Pb biomarkers have been demonstrated to relate to maternal Pb biomarkers as reported in the 2006 Pb AQCD (U.S. EPA, 2006b). Kordas et al. (2010) observed that maternal hair Pb concentration was a statistically significant predictor of child hair Pb concentration ($\beta = 0.37 \pm 0.07$, $p < 0.01$). Elevated blood Pb levels among mothers present a potential exposure route to their children in utero or through breast milk; see Miranda et al. (2010).

5.2.1.2 Older Adulthood

Blood Pb levels tend to be higher in older adults compared with the general adult population, as described in the 2006 Pb AQCD (U.S. EPA, 2006b). Table 5-2 presents 2009-2010 NHANES data broken down by age group and shows that blood Pb levels were highest in the among participants 60 years old or older, in comparison with adults aged 20-59 years and with adolescents. In a study of blood Pb and saliva Pb in a mostly female population in Detroit, Nriagu et al. (2006) found that age was a statistically significant positive predictor of blood Pb ($p < 0.001$). Average blood Pb levels among 14- to 24-year-old subjects was 2.60 ± 0.16 μg/dL compared with 4.29 ± 0.56 μg/dL among subjects aged 55 years or older. Higher average and median levels among older adults could potentially be due to a shared experience of higher historical Pb exposures stored in bone in conjunction with remobilization of stored Pb during bone loss (Section 3.2).

Theppeang et al. (2008b) studied Pb concentrations in the blood, tibia, and patella of subjects age 50-70 as part of the Baltimore Memory Study. They found a statistically significant relationship between age and tibia Pb ($\beta = 0.37$, $p < 0.01$ in a model including age, race/ethnicity, Yale energy index, and 2 diet variables; $\beta = 0.57$, $p < 0.01$ in a model including age, sex, and an interaction term for sex and age, which was also statistically significant at $p = 0.03$). Theppeang et al. (2008b) also noted that patella Pb concentrations were also positively associated with age, although the authors did not present the data or significance levels. A statistically significant relationship was not observed between the log-transform of blood Pb and age ($\beta = 0.007$, $p = 0.11$), although the age range of subjects may not have been sufficient to discern a difference in blood Pb level.

Miranda et al. (2010) observed that older pregnant women (ages 30-34 years and 35-39 years) had statistically significant higher odds of having greater blood Pb levels than younger pregnant women (25- to 29-year-olds) in the reference age category. These results could be related to a historical component to Pb exposure among mothers. These findings were also consistent with observations that Pb storage in bones increased with age before subsequent release with bone loss occurring during pregnancy, as described in Section 3.2 and summarized in Section 5.1.

5.2.2 Sex

The AQCD (U.S. EPA, 2006b) described several studies showing higher Pb biomarker levels in male adults compared with female adults. The 2009-2010 NHANES showed that overall, males have significantly higher blood Pb levels (average: 1.75 µg/dL) than females (average: 1.25 µg/dL) ($p < 0.0005$). Among adults aged 20-59 years, average blood Pb levels were 64% higher for males compared with females ($p < 0.0005$). Among adults 60 years or older, average blood Pb levels were 30% higher for males compared with females ($p < 0.0005$) (NCHS, 2010). In their study of Pb burden among Baltimore adults aged 50-70 years, Theppeang et al. (2008b) observed that average blood Pb levels were statistically significantly higher ($p < 0.01$) among men (4.4 µg/dL) than women (3.1 µg/dL). For average tibia Pb levels, Theppeang et al. (2008b) noted no difference ($p = 0.12$) between men (18.0 µg/g) and women (19.4 µg/g).

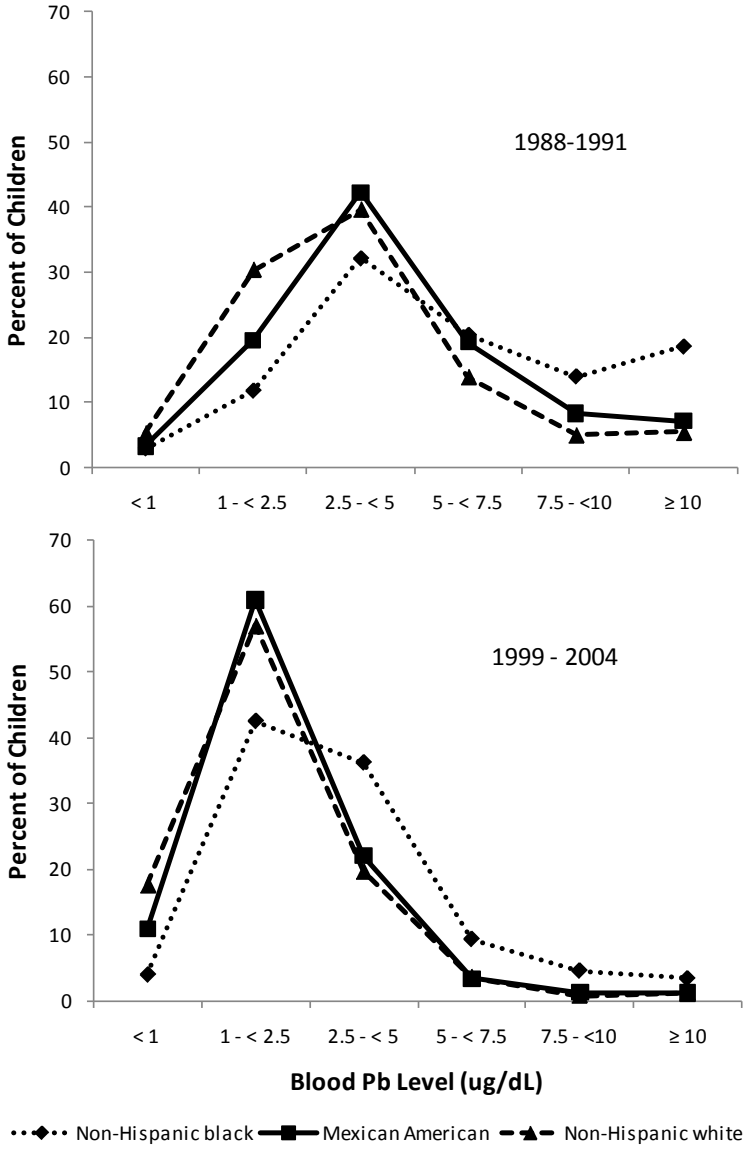
Among U.S. children, the 2009-2010 NHANES data showed that blood Pb levels were higher among girls than boys for the 1- to 5-years age group (Table 5-2). Blood Pb levels became slightly higher among boys for the 6- to 11-years age group, and levels were substantially higher among adolescent males than females 12- to 19-years old. The 2009-2010 NHANES data suggest that sex-based differences in blood Pb levels are not substantial until adolescence.

5.2.3 Race and Ethnicity

Higher blood Pb and bone Pb levels among African Americans have been well documented (U.S. EPA, 2006b). Structural equation model results by Lanphear and Roghmann (1997) demonstrated not just elevations in blood Pb among African Americans but also significant associations between blood Pb and race. Recent studies are consistent with those previous findings. For instance, Levin et al. (2008) and Jones et al. (2009a) both analyzed NHANES survey data to examine trends in childhood blood Pb levels. Data from the Jones et al. (2009a) study, using NHANES data (NCHS, 2009, 2008) from 1988-1991 and 1999-2004 are shown in Figure 5-1. The authors found that differences among children from different racial/ethnic groups with regard to the percentage with blood Pb levels ≥ 2.5 µg/dL over the period 1999-2004, have decreased since the period of 1988-1991. The non-Hispanic black group still had higher percentages with blood Pb levels ≥ 2.5 µg/dL compared with non-Hispanic whites and Mexican Americans, with large observable differences for blood Pb levels between 2.5 and <10 µg/dL. It is notable that the distributions of blood Pb levels among Mexican American and non-Hispanic white children were nearly identical in the 1999-2004 dataset. Theppeang et al. (2008b) also explored the effect of race and ethnicity on several

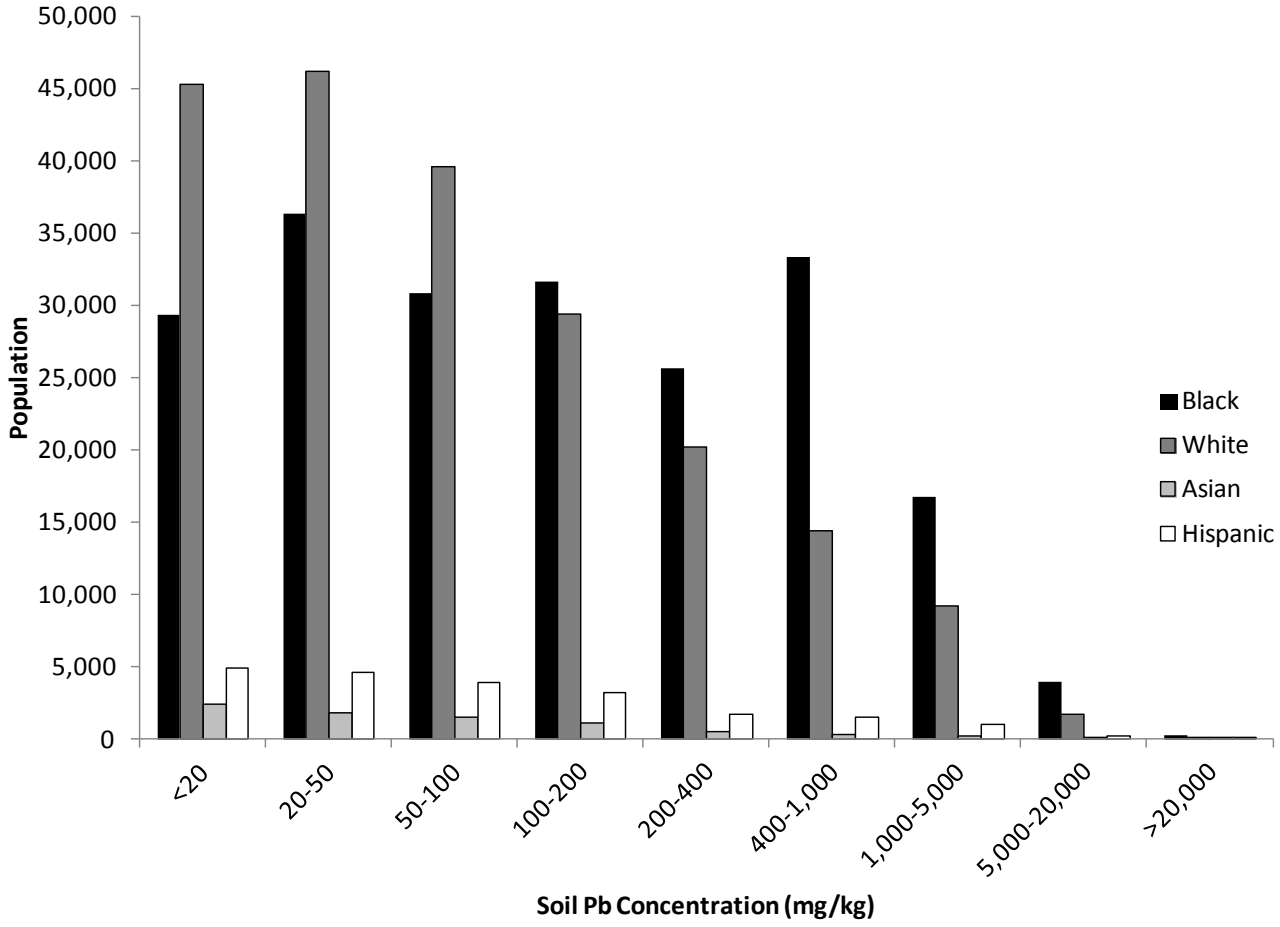
Pb biomarkers in a study of older adults living in Baltimore, MD. They observed a statistically significant difference between African American (AA) and Caucasian (C) subjects with respect to tibia Pb (AA: 21.8 µg/g, C: 16.7 µg/g, $p < 0.01$) but not patella Pb (AA: 7.1 µg/g, C: 7.1 µg/g, $p = 0.46$) or blood Pb levels (AA: 3.6 µg/dL, C: 3.6 µg/dL, $p = 0.69$). Greater tibia (but lower patella) Pb levels may indicate greater historical exposure among African Americans compared to Caucasians in the Baltimore population studied by Theppeang et al. (2008b).

Differences in potential exposure among ethnic and racial groups have also been noted in a study in the greater metropolitan New Orleans area. Campanella and Mielke (2008) found that, in Census blocks where surface soil Pb levels were less than 20 mg/kg, the population was 36% black, 55% white, 3.0% Asian, and 6.0% Hispanic, based on the 2000 Census, with the percentage based on the total number living in Census blocks with the same soil Pb levels. In contrast, they found that for Census blocks in which soil Pb levels were between 1,000 and 5,000 mg/kg, the population was 62% black, 34% white, 1% Asian, and 4% Hispanic (Figure 5-2) , although the total population size generally declined with soil Pb concentration, with the Census blocks with soil Pb of 1,000-5,000 mg/kg having less than half the population of that in the <20 mg/kg blocks. As described in Section 5.2.4, the differences observed by Campanella and Mielke (2008) may also be attributable to SES factors, or SES may be a confounding factor in the relationship between Pb soil levels and race/ethnicity of nearby residents.



Note: from the NHANES survey, 1988-1991 (top) and 1999-2004 (bottom).
Data used with permission of the American Academy of Pediatrics, Jones et al. (2009a)

Figure 5-1 Percent distribution of blood Pb levels by race/ethnicity among U.S. children (1-5 years).



Note: By Census 2000 race/ethnicity demographic groups.
Source: Data used with permission of Springer Science; Campanella and Mielke (2008).

Figure 5-2 Soil Pb concentration exposure among the population of three parishes within greater metropolitan New Orleans.

5.2.4 Socioeconomic Status (SES)

Socioeconomic factors have sometimes been associated with Pb exposure biomarkers. Previous results reported in the 2006 Pb AQCD found negative associations between income or other SES metrics and blood Pb, although these relationships were not always statistically significant (U.S. EPA, 2006b). Nriagu et al. (2006) performed a multiple regression analysis of blood Pb and saliva Pb levels on various socioeconomic, demographic, and exposure variables among an adult population in Detroit, Michigan. Blood and saliva Pb were both used as indicators of Pb in unbound plasma that is available to organs. Nriagu et al. (2006) found that education ($p < 0.001$), income ($p < 0.001$), and employment status ($p = 0.04$) were all statistically significant predictors of blood Pb levels, with blood Pb decreasing with some scatter as education and income level increased. Statistically significant relationships were also reported by Nriagu et al. (2006) for saliva Pb level with respect to education ($p < 0.001$), income ($p < 0.001$), and employment ($p = 0.06$). However, the highest educational attainment and income categories had higher saliva Pb levels compared with other groups; Nriagu et al. (2006) attributed these inconsistencies to small sample sizes among the high educational attainment and income categories.

On a national level, the difference in blood Pb levels that have historically been seen to exist between different income levels has been decreasing. For example, Levin et al. (2008) cited 1991-1994 NHANES data [analyzed in Pirkle et al. (1994)] that the percentage of children aged 1-5 years with blood Pb levels ≥ 10 μg/dL was 4.5% for the lowest income group compared with 0.7% for the highest income group. Levin et al. (2008) also analyzed data from the 1999-2002 NHANES and found no statistically significant difference between the percent of children with blood Pb levels above 10 μg/dL for Medicaid-enrolled children (1.7%) compared with non-enrolled children (1.3%). However, Medicaid-enrolled children did have higher median blood Pb levels (2.6 μg/dL) compared to children not enrolled in Medicaid (1.7 μg/dL). Adding data for 2003-2004 to the analysis (i.e., for 1999-2004), widened the difference between Medicaid enrolled and non-enrolled children with regard to percentage having blood Pb levels ≥ 10 μg/dL (1.9% versus 1.1%), but the difference was still not statistically significant ($p > 0.05$) and median blood Pb levels for the two groups did not change (Levin et al., 2008). Likewise, Jones et al. (2009a) analyzed blood Pb levels with respect to poverty-income ratio (PIR), which is the ratio of family income to the poverty threshold appropriate for a given family size. They found statistically significant differences in median blood Pb for PIR ≤ 1.3 compared with PIR >1.3. The percentage of 1- to 5-year-old children having blood Pb ≥ 10 μg/dL was higher for PIR ≤ 1.3 (1.8 versus 0.8); however, this difference was not statistically significant. Additionally, in residential areas of metropolitan New Orleans with soil concentrations below 20,000 mg/kg, Campanella and Mielke (2008)

observed a linear increase in surface soil Pb concentration with decreasing median household income, suggesting a relationship of potential exposure with household income. The census block-averaged median household income in areas with soil Pb between 2.5 and 20 mg/kg was $40,000 per year, while the corresponding median income in areas with soil Pb between 5,000 and 20,000 mg/kg was $24,000 per year. The highest soil concentrations (20,000 mg/kg and above) was associated with a median income of $27,000.

5.2.5 Proximity to Pb Sources

Air and soil Pb concentrations are higher in some industrialized and urbanized areas, as described in Sections 2.2, 2.3, 2.5, and 3.1, as a result of historical and contemporary Pb sources. The highest air Pb concentrations measured using the Pb-TSP monitoring network have been measured at monitors located near sources emitting Pb. Elevated soil Pb concentrations have also been measured in urbanized areas compared with less urbanized or rural locations (Filippelli et al., 2005). Air Pb concentrations exhibit high spatial variability even at low concentrations (~0.01 μg/m^3) (Martuzevicius et al., 2004). Proximity to an industrial source likely contributes to higher Pb exposures, as described in the 2006 Pb AQCD (U.S. EPA, 2006b) for several studies of Superfund and other industrial sites. This is consistent with the observation of higher air concentrations at source oriented Pb monitoring sites compared with non-source oriented sites in the 2008-2010 data presented in Section 2.5.

Jones et al. (2010) found that neonates born near a Pb-contaminated hazardous waste site had significantly higher umbilical cord blood Pb levels (median: 2.2 μg/dL [95% CI: 1.5, 3.3 μg/dL]) compared with a reference group of neonates not living near a potentially contaminated site (median: 1.1 μg/dL [95% CI: 0.8, 1.3 μg/dL]), suggesting that Pb-contaminated hazardous waste sites contribute to neonatal Pb levels. The population studied in Jones et al. (2010) was 88% African American; 75% had a high school degree or equivalent, while 20% had a college degree and 5% attended but did not graduate from high school. However, Jones et al. (2010) did not analyze covariation between exposure and maternal characteristics, so it cannot be determined if differences in characteristics among the maternal groups (which did and did not report nearby hazardous waste sites) confounded these results.

Studies have suggested that concentration of Pb in soil, a potential exposure media, is related to land use type and historical sources, as described in Section 2.6.1. For instance, Wu et al., (2010) observed that bioavailable Pb concentrations in Los Angeles surface soil samples were significantly associated with traffic-related variables and parcel age

(i.e., length of time since the parcel was first developed), with parcel age being a highly significant predictor of bioavailable soil Pb in most models ($p < 0.0001$). Zahran et al. (2010) observed that surface soil Pb levels in 46 Census tracts of metropolitan New Orleans dropped following Hurricanes Katrina and Rita, from 330 mg/kg to 200 mg/kg (averages of median measurements across all Census tracts for 2000 and 2006) and attributed this observation to coverage by relatively cleaner river sediments. Blood Pb levels obtained from children (ages 0-6 years) also declined subsequent to the hurricanes; statistical modeling of the changes in soil and blood Pb estimated the decline to be 1.55 µg/dL for each 1% reduction in soil Pb ($p \leq 0.05$).

5.2.6 Residential Factors

Findings from a recent study of the association between blood Pb and housing factors by Dixon et al. (2009), which analyzed data from the NHANES national survey for 1999-2004, are consistent with those from previous studies presented in the 2006 Pb AQCD that observed positive associations between increased blood Pb and increased house dust Pb levels (U.S. EPA, 2006b; Lanphear et al., 1998; Laxen et al., 1987). Dixon et al. (2009) used NHANES data from 1999-2004 to perform a linear regression of blood Pb among children 12-60 months old on several factors including year of home construction, floor surface condition, floor dust Pb level, windowsill dust Pb level, and renovation in homes built before 1978. They found an inverse association between blood Pb (log transformed) and residence in homes being built after 1950 ($p = 0.014$). Blood Pb was positively associated with windowsill Pb level ($p = 0.002$), dust Pb level ($p < 0.001$), and occurrence of renovation in pre-1978 homes ($p = 0.045$). Detailed results of this regression are shown in Table 5-4. As part of the same study, Gaitens et al. (2009) performed a regression analysis of floor dust Pb (PbD) and windowsill dust Pb on several factors. Floor dust Pb (log transformed) was positively associated with the following housing-related factors: not smooth/cleanable floor surface ($p < 0.001$), windowsill dust Pb (log transformed) ($p < 0.001$), and older year of construction ($p < 0.001$). Windowsill dust Pb (log transformed) was positively associated with the following housing-related factors: older year of construction ($p < 0.001$), not smooth/cleanable window surface (0.001) and deteriorated indoor paint ($p = 0.028$).

Table 5-4 Regression of log-transformed blood Pb level of children 12-60 months old on various factors related to housing condition, from 1999-2004 NHANES dataset.

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

Table 5-4 (Continued): Regression of log-transformed blood Pb level of children 12-60 months old on various factors related to housing condition, from 1999-2004 NHANES dataset.

Variables	Overall *p*-value	Levels[a]	Estimate (SE)	*p*-Value
log Window-sill PbD	0.002	Intercept for missing	0.053 (0.040)	0.186
		Slope	0.041 (0.011)	<0.001
Home-apartment type	<0.001	Intercept for missing	–0.064 (0.097)	0.511
		Mobile home or trailer	0.127 (0.067)	0.066
		One family house detached	–0.025 (0.046)	0.596
		One family house attached	0.000	—
		Apartment (1–9 units)	0.069 (0.060)	0.256
		Apartment (≥ 10 units)	–0.133 (0.056)	0.022
Anyone smoke inside the home	0.015	Missing	0.138 (0.140)	0.331
		Yes	0.100 (0.040)	0.015
		No	0.000	—
log Cotinine concentration (ng/dL) in blood	0.004	Intercept for missing	–0.150 (0.063)	0.023
		Slope	0.039 (0.012)	0.002
Window cabinet or wall renovation in a pre-1978 home	0.045	Missing	–0.008 (0.061)	0.896
		Yes	0.097 (0.047)	0.045
		No	0.000	—

[a]Children: N = 2,155 (age 10-60 months); R^2 = 40%

[b]Includes the 50 states and the District of Columbia

Source: Dixon et al. (2009).

Renovation activities on older homes have been shown to produce excess Pb dust concentrations. Gaitens et al. (2009) performed a regression analysis on dust Pb concentrations from 1994-2004 NHANES on demographic and housing variables and found that renovation of windows, cabinets, or walls in a pre-1950 home was positively associated with floor dust Pb concentration ($p < 0.001$). Paint scraping within the last twelve months was positively associated with windowsill dust Pb concentration ($p = 0.053$). Dixon et al. (2009) performed a regression analysis on log-transformed blood Pb levels from NHANES (1999-2004) on several demographic and housing variables and found that renovation of windows, cabinets, or walls in pre-1978 homes was positively associated with blood Pb concentration ($p = 0.045$). A case study by Mielke et al. (2001) reports on elevated indoor and outdoor dust Pb levels at two houses where exterior paint has been either power sanded (without confinement of released material) or hand scraped (with collection of released material) to prepare for repainting. The latter approach appeared to yield lower dust Pb levels, although given the extremely limited dataset, conclusions are uncertain. In an occupational study of men performing home renovations in the U.K., window renovation and wood-stripping workers specializing in renovation of old houses had significantly higher median blood Pb levels compared with all workers in

similar occupations (wood strippers: 37 µg/dL; window renovators: 32 µg/dL; all workers: 13.7 µg/dL; p <0.001) (Mason et al., 2005).

5.3 Factors Potentially Related to Increased Risk of Pb-Induced Health Effects

This section evaluates factors examined in recent studies as effect measure modifiers that potentially increase the risk of various Pb-related health effects. There was limited evidence from the 2006 Pb AQCD (U.S. EPA, 2006b) for many of potential at-risk factors described below. Where available, information on conclusions regarding at-risk populations from the 2006 Pb AQCD is included in the subsections.

5.3.1 Age

Below is information from epidemiologic and toxicological studies regarding studies of increased risk for Pb-related health effects among children and older adults. Other age groups, such as adolescents, have not been evaluated here, if they were not part of stratified studies of lifestage.

5.3.1.1 Childhood

According to the 2010 Census, 24.0% of individuals living in the U.S. were under the age of 18, with 6.5% aged 0-4 years and 17.5% aged 5-17 years (Howden and Meyer, 2011). It is recognized that Pb can cross the placenta and affect the developing nervous system of the fetus (Sections 3.2.2.4 and 4.3) and there is strong evidence of increased risk to the neurocognitive effects of Pb exposure during several lifestages throughout gestation, childhood, and into adolescence (for more detail, Section 4.3). However, most recent studies among children do not have adequate comparison groups between children of various age groups or between children and adults, and were therefore only presented in Chapter 4. Overall, early childhood has been demonstrated to be a lifestage of increased risk for Pb-related health effects.

A study including multiple U.S. locations examined associations of blood Pb levels with various immune parameters among individuals living near Pb industrial sites and matched controls (Sarasua et al., 2000). For several of these endpoints, the association in the youngest group (ages 6-35 months) and the oldest group (ages 16-75 years) were in opposite directions. For example, among children ages 6-35 months, the associations

between blood Pb levels and Immunoglobulin A (IgA), Immunoglobulin M (IgM), and B-cell abundance were positive, whereas the associations among 16-75 year olds were negative. The opposite associations were also present for T cell abundance. Ig antibodies, which are produced by activated B cells, are important mediators of the humoral immune response to antigens. T cells are important mediators of cell-mediated immune responses that involve activation of other immune cells and cytokines. These findings by Sarasua et al. (2000) indicate that very young children may be at increased risk for Pb-associated activation of humoral immune responses and perturbations in cell-to-cell interactions that underlie allergic, asthma, and inflammatory responses (for more information, see Sections 4.6.2.1 and 4.6.3).

A study among Lebanese children examined the association between blood Pb levels and transferrin saturation (TS) less than 12% (a measure of Fe-deficiency with or without accompanying anemia) and Fe (iron)-deficiency anemia (IDA) (Muwakkit et al., 2008). A positive association was detected for blood Pb levels ≥ 10 µg/dL and both TS less than 12% and IDA among children aged 11-23 months old; however, null associations were observed among children 24-35 months old. Calculations were not performed for children aged 36-75 months because there were no children in the highest Pb group (≥ 10 µg/dL) with either TS <12% or IDA. The authors noted that it is difficult to know whether the Pb levels were "a cause or a result of" IDA levels since previous studies linked Fe deficiency with Pb toxicity.

Overall evidence indicates early childhood as a lifestage of increased risk for Pb-related health effects. Both epidemiologic studies summarized above reported associations among the youngest age groups, although different age cut-points were used with one study including only infants 35 months of age and younger. Toxicological studies provide support for increased health effects of Pb among younger age groups. Toxicological studies have reported that younger animals, whose nervous systems are developing (i.e., laying down and pruning neuronal circuits) and whose junctional barrier systems in the brain (i.e., the blood brain barrier) and GI system (i.e., gut closure) are immature, are more at risk from the effects Pb exposure (Rice and Barone, 2000; Landrigan et al., 1999). In sum, there are consistent findings, coherent across disciplines that adequate evidence exists to conclude that children are an at-risk population. There are insufficient data to identify specific critical windows of exposure for Pb-related effects.

5.3.1.2 Older Adulthood

The number of Americans over the age of 65 will be increasing in upcoming years (estimated to increase from 12.4% of the U.S. population to 19.7% between 2000 to

2030, which is approximately 35 million and 71.5 million individuals, respectively) (SSDAN CensusScope, 2010; U.S. Census Bureau, 2010). As of the 2010 Census, 7.0% of the U.S. population were ages 65-74, 4.2% were 75-84, and 1.7% were age 85 and older (Werner, 2011). Overall, inconsistent results are reported for age-related modification of the association between Pb and mortality. There is limited evidence for the associations between Pb and other health effects among older adults.

A study using the NHANES III cohort examined blood Pb levels and mortality among individuals less than 60 years old and individuals 60 years and older (Menke et al., 2006). Positive hazard ratios were observed in both age groups but the hazard ratios were greater in those less than 60 years old. The interaction terms were not statistically significant. A similar study using the NHANES III cohort examined the relationship between blood Pb levels and mortality from all-cause, cardiovascular disease, and cancer broken down into more specific age groups (Schober et al., 2006). Point estimates were elevated for the association comparing blood Pb levels ≥ 10 µg/dL to blood Pb levels <5 µg/dL and all-cause mortality for all age groups (40-74, 75-84, and 85+ year olds), although the association for 75-84 year olds did not reach statistical significance. The association was also present when comparing blood Pb levels of 5-9 µg/dL to blood Pb levels <5 µg/dL among 40-74 year olds and 75-84 year olds, but not among those 85 years and older. None of the associations between blood Pb and cardiovascular disease-related mortality reached statistical significance but the point estimates for cardiovascular disease-related mortality comparing blood Pb levels ≥ 10 µg/dL to blood Pb levels <5 µg/dL were elevated among all age groups. Finally, the association between blood Pb levels ≥ 10 µg/dL and cancer mortality was positive among those 40-74 years old and 85 years and older but the association was null for those 75-84 years old. Among 75-84 year olds the association was positive comparing blood Pb levels of 5-9 µg/dL to <5 µg/dL. The other age groups had similar point estimates but the associations were not statistically significant.

A study using the Normative Aging Study cohort reported an interaction between Pb and age (Wright et al., 2003). The inverse association between age and cognitive function was greater among those with high blood or patella Pb levels. Effect estimates were in the same direction for tibia Pb but the interaction was not statistically significant.

Finally, a study of current and former Pb workers reported that an interaction term of Pb and age (dichotomous cutpoint at 67th percentile but exact age not given) examined in models of Pb (measured from blood and patella) and blood pressure was not statistically significant (Weaver et al., 2008). Thus, no modification by age was observed in this study of Pb and blood pressure.

Toxicological studies have demonstrated Pb-related health effects among older populations. The kidneys of older animals appear to be more at-risk for Pb-related health effects from the same dose of Pb (i.e., continuous 50 mg/L Pb acetate drinking water) than younger animals (Berrahal et al., 2011). Increased risk related to older age is also observed for effects on the brain. Recent studies have demonstrated the importance of Pb exposure during early development in promoting the emergence of Alzheimer's like pathologies in aged animals. Development of pathologies of old age in brains of aged animals that were exposed to Pb earlier in life has been documented in psychopathological effects in adults (mice and monkeys), (for more details see Section 4.3.10.1). These pathologies include the development of neurofibrillary tangles and increased amyloid precursor protein and its product beta-amyloid (Basha et al., 2005; Zawia and Basha, 2005). Some of these findings were seen in animals that no longer had elevated blood Pb levels.

In summary, results for age-related modification of the association between Pb and mortality had mixed results. Limited evidence was available for the associations between Pb and cognitive function or other health effects among older adults. Toxicological studies have shown increases in Pb-related health effects by age that may be relevant in humans. Future studies will be instrumental in understanding older age as a factor that potentially affects the risk of Pb-related outcomes.

5.3.2 Sex

The distribution of males and females in the U.S. is similar. In 2010, 49.2% of the U.S. population was male and 50.8% was female. The distribution of sex varied by age with a greater prevalence of females in older age groups compared to males (Howden and Meyer, 2011). The 2006 Pb AQCD reported that boys are often found to have higher blood Pb levels than girls, but findings were "less clear" regarding differences in Pb-related health effects between males and females (U.S. EPA, 2006b). Recent studies examining effect measure modification by sex have also reported inconsistent findings.

Multiple epidemiologic studies have examined Pb-related effects on cognition stratified by sex. In previous studies using the Cincinnati Lead Study cohort, Dietrich et al. (1987) and Ris et al. (2004) observed interactions between blood Pb (prenatal and postnatal) and sex; associations of prenatal and postnatal blood Pb and subsequent decrements in memory, attention, and visuoconstruction were observed only among male adolescents. More recently, Wright et al. (2008) examined early life blood Pb levels and criminal arrests in adulthood. The risks attributable to Pb exposure were greater among males than

females. Additionally, the association between childhood blood Pb levels and adult gray matter volume loss was greater among males than females (Cecil et al., 2008). In an expanded analysis of the developmental trajectory of childhood blood Pb levels on adult gray matter, researchers found that associations between yearly mean blood Pb levels and volume of gray matter loss were more pronounced in the frontal lobes of males than females (Brubaker et al., 2010). Multiple studies were also conducted in Port Pirie, Australia that examined blood Pb levels at various ages throughout childhood and adolescence (Tong et al., 2000; Baghurst et al., 1992; McMichael et al., 1992). These studies observed Pb effects on cognition deficits were stronger in girls throughout childhood and into early adolescence. A study in Poland also investigated the association between umbilical cord blood Pb levels and cognitive deficits and reported a positive association for boys at 36 months but not for girls (Jedrychowski et al., 2009a). No association was detected for boys or girls at 24 months.

An epidemiologic study examined the association between concurrent blood Pb levels and kidney function among 12-20 year olds using the NHANES III study cohort (Fadrowski et al., 2010). The results were stratified by sex and no effect measure modification was apparent.

Similarly, a study of current and former Pb workers examined an interaction term between sex and Pb for the study of blood Pb and blood pressure (Weaver et al., 2008). No modification by sex was present.

Epidemiologic studies have also been performed to assess differences between males and females for Pb-related effects on various biomarkers. A study comprised mostly of females reported positive associations between blood Pb and total immunoglobulin E (IgE) for women not taking hormone replacement therapy or oral contraceptives (Pizent et al., 2008). No association was reported in males, but other associations, such as bronchial reactivity and reactive skin prick tests were observed in the opposite of the expected direction, which questions the validity of the results among the male study participants. Analysis of an NHANES dataset detected no association between blood Pb levels and inflammatory markers (Songdej et al., 2010). Although there was no clear pattern, a few of the associations were positive between blood Pb and C-reactive protein for males but not females. A study of children living at varying distances from a Pb smelter in Mexico reported that blood Pb was associated with increased release of superoxide anion from macrophages, which was greater among males than females (Pineda-Zavaleta et al., 2004).

Epidemiologic investigations of cancer have also examined the associations by sex. A study of the association between occupational exposure to Pb and brain tumors reported no sex-specific associations for gliomas, but a positive association for cumulative Pb

exposure and meningiomas for males but not females (Rajaraman et al., 2006). An ecologic analysis of Pb pollution levels and cancer incidence among children reported weak correlations overall and the weak correlations were more apparent among males, whereas no correlation was observed among females (Absalon and Slesak, 2010).

A study of all-cause and cardiovascular mortality using the NHANES III cohort reported no modification of the association between blood Pb and all-cause or cardiovascular mortality by sex (Menke et al., 2006). This did not differ among women when classified as pre- or post-menopausal.

Toxicological studies have also reported sex differences in Pb-related effects to various organ systems. Donald et al. (1986) reported a different time course of enhanced social investigatory behavior between male and female mice exposed to Pb. In a subsequent publication, Donald et al. (1987) showed that non-social behavior in mice decreased in females and increased in males exposed to Pb. Males also had a shorter latency to aggression with Pb treatment versus controls. Pb affected mood disorders differently for males and females. Behavioral testing in rats showed males experienced emotional changes and females depression-like changes with Pb exposure (de Souza Lisboa et al., 2005). In another study, gestational exposure to Pb impaired memory retrieval in male rats at all 3 doses of Pb exposure; memory retrieval was only impaired in low-dose female rats (Yang et al., 2003). Sex-specific differences in mice were also observed for gross motor skills; at the lowest Pb dose, balance and coordination were most affected among males (Leasure et al., 2008).

Pb and stress are co-occurring factors that act in a sex-divergent manner to affect behavior, neurochemistry, and corticosterone levels. Pb and stress act synergistically to affect fixed interval operant behavior and corticosterone in female rat offspring. Virgolini et al. (2008a) found that effects on the offspring's central nervous system by developmental Pb exposure (maternal exposure and transferred to the offspring through lactation) were enhanced by combined maternal and offspring stress and females were most at risk. Behavioral related outcomes after gestational and lactational Pb exposure (with and without stress) exhibited sex-differences in exposed offspring (Virgolini et al., 2008b). Pb-induced changes in brain neurochemistry, with or without concomitant stress exposure, are complex with differences varying by brain region, neurotransmitter type, and sex of the animal.

The brain is known to have a sexually dimorphic area in the hypothalamus, termed the sexually dimorphic nucleus (SDN). Lesions in this area affect sex-specific phenotypes including behavior. Across species the SDN has a greater cell number and larger size in males versus females. This sexually dichotomous area is especially vulnerable to perturbation during fetal life and the early postnatal period. This may be one area of the

brain that could explain some of the sexually dichotomous effects that are seen with Pb exposure. One study supporting this line of thought showed that high-dose in utero Pb exposure (pup blood Pb level 64 µg/dL at birth) induced reductions in SDN volume in 35% of Pb-exposed male rats (McGivern et al., 1991). Interestingly, another chemical that is known to cause a hypothalamic lesion in this area, monosodium glutamate, is associated with adult onset obesity among mice (Olney, 1969); adult onset obesity is seen in the Pb literature.

Obesity in adult offspring exposed to low-dose Pb in utero was reported for male but not female mice (Leasure et al., 2008). Obesity was also found in male rat offspring exposed in utero to high doses of Pb that persisted to 5 weeks of age/end of the study, but among female rats, body weight remained elevated over controls only to 3 weeks of age (Yang et al., 2003). Additionally, low-dose Pb exposure induced retinal decrements in exposed male mice offspring (Leasure et al., 2008).

A toxicological study of Pb and antioxidant enzymes in heart and kidney tissue reported that male and female rats had differing enzymatic responses, although the amount of Pb in the heart tissue or the disposition of Pb also varied between males and females (Sobekova et al., 2009; Alghazal et al., 2008a). The authors reported these results could be due to greater deposition of Pb in female rats or greater clearance of Pb by males (Sobekova et al., 2009).

Multiple associations between Pb and various health endpoints have been examined for effect measure modification by sex. Some studies reported differences in the risk of Pb-related outcomes by sex, but did not consistently demonstrate whether male or female sex was associated with a greater risk; studies on cognition from the Cincinnati Lead Study cohort and a study in Poland reported males to be an at-risk population, whereas studies from Australia pointed to females as an at-risk population. A difference in sex is supported by toxicological studies. Further research is needed to confirm the presence or absence of sex-specific associations between Pb and various health outcomes and to determine in which sex the associations are greater.

5.3.3 Genetics

The 2006 Pb AQCD stated that, "genetic polymorphisms in certain genes have been implicated as influencing the absorption, retention, and toxicokinetics of Pb in humans" (U.S. EPA, 2006b). The majority of discussion there focused on the aminolevulinate dehydratase (ALAD) and vitamin D receptor (VDR) polymorphisms. These two genes, as well as additional genes examined in recent studies, are discussed below. The presence of

ALAD variants is associated with an increase in Pb-related health outcomes, but there is limited information for other genes.

5.3.3.1 Aminolevulinate Dehydratase

The aminolevulinate dehydratase (ALAD) gene encodes for an enzyme that catalyzes the second step in the production of heme and is also the principal Pb-binding protein (U.S. EPA, 2006b). Studies have examined whether ALAD variants altered associations between Pb and various health effects.

Associations between Pb and brain tumors observed in an epidemiologic study varied by ALAD genotype status (Rajaraman et al., 2006). Positive associations between Pb exposure (determined via interview about occupational exposures) and meningioma were reported among ALAD2 individuals, but this association was not found among individuals who had the ALAD1 allele. No associations were observed between Pb and glioma regardless of ALAD genotype.

Studies investigating the association between Pb levels and cognitive function have also examined modification by ALAD polymorphisms. The evidence is provided by an NHANES analysis (Krieg et al., 2009) as well as multiple analyses from the NAS cohort examining different tests of cognitive function (Rajan et al., 2008; Weuve et al., 2006). In the study using a cohort from NHANES III, for several indices of cognitive function, associations with concurrent blood Pb levels were more pronounced in groups with CC and CG ALAD genotypes (i.e., ALAD2 carriers) (Krieg et al., 2009). In the NAS cohort of men, Weuve et al. (2006) found that concurrent blood Pb level but not bone Pb level was associated with a larger decrease in a test of general cognitive function among ALAD2 carriers. Another NAS study examined functioning of specific cognitive domains (e.g., vocabulary, memory, visuospatial skills) and found variable evidence for effect modification by ALAD genotype across tests (Rajan et al., 2008). For example, among ALAD2 carriers, concurrent blood Pb level was associated with a more pronounced decrease in vocabulary score but less pronounced decrease in a memory index and no difference in the associations with other cognitive tests. For tibia and patella Pb levels, ALAD genotype was found to modify associations with different tests, for example, executive function and perceptual speed. It is not clear why the direction of effect modification would vary among different cognitive domains. The limited number of populations examined, and the different cognitive tests performed in each study, make it difficult to conclusively summarize findings for effect modification by ALAD variants.

However, in the limited available body of evidence, blood and bone Pb levels were generally associated with lower cognitive function in ALAD2 carriers.

A study of current and former workers exposed to Pb examined the association between blood Pb and blood pressure and reported no modification by ALAD genotype (Weaver et al., 2008). However, another study of blood Pb and blood pressure reported interactions between blood Pb and ALAD, but this varied by race/ethnicity (non-Hispanic white, non-Hispanic black, and Mexican American) (Scinicariello et al., 2010). In this study, non-Hispanic white carriers of the ALAD2 genetic variant in the highest blood Pb quartile had a higher risk of hypertension compared with ALAD1 homozygous individuals. The association between ALAD variants and hypertension did not vary within any quartiles of blood Pb for non-Hispanic blacks or Mexican-Americans, but the precision of estimates was low.

Individuals with ALAD2 variants had greater associations between Pb and kidney effects; among those with the variant, higher Pb was associated with higher glomerular filtration measures (Weaver et al., 2006; Weaver et al., 2005b; Weaver et al., 2003b). A study of workers at a battery plant storage facility in China reported workers with the ALAD2 allele demonstrated greater associations between blood Pb levels and renal injury (Gao et al., 2010a). Another study of renal function among Pb workers in Asia also reported greater associations between blood Pb concentrations and renal function by ALAD, especially at high blood Pb levels (Chia et al., 2006).

5.3.3.2 Vitamin D Receptor

The vitamin D receptor (VDR) is a regulator of calcium absorption and metabolism. A recent study of the NHANES III population examined the association between blood Pb levels and various neurocognitive tests with assessment of effect measure modification by SNPs and haplotypes of VDR (Krieg et al., 2010). The results were varied, even among specific SNPs and haplotypes, with some variants being associated with greater modification of the relationship between Pb and one type of neurocognitive test compared to the modification of the relationship between Pb and other neurocognitive tests. In an epidemiologic study of blood Pb levels and blood pressure among a group of current and former Pb-exposed workers, no modification was reported by VDR (Weaver et al., 2008).

5.3.3.3 Methylenetetrahydrofolate reductase

Methylenetetrahydrofolate reductase (MTHFR) catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which in turn, is involved in homocysteine remethylation to the amino acid methionine. A study in Mexico of the association between Pb and Bayley's Mental Development Index (MDI) score at 24 months reported no effect measure modification by MTHFR 677T allele (Pilsner et al., 2010). Another study in Mexico examined the association between maternal Pb and birth weight (Kordas et al., 2009). No modification of the Pb-birth weight association by MTHFR was observed.

5.3.3.4 Hemochromatosis

The hemochromatosis (HFE) gene encodes a protein believed to be involved in Fe absorption. A difference was observed between the association of tibia Pb levels and cognitive function for men with and without HFE allele variants (Wang et al., 2007a). No association between tibia Pb and cognitive function was present for men with HFE wildtype, but a decline in function was associated with tibia Pb levels among men with any HFE allele variant. A study of bone Pb levels and HFE reported no difference in effect estimates for bone Pb and pulse pressure between different HFE variants and HFE wild-type (Zhang et al., 2010a). An interaction was observed between an HFE variant in mothers and maternal tibia Pb in a study of maternal Pb and birth weight (Cantonwine et al., 2010b). The inverse association between maternal tibia Pb levels and birth weight was stronger for those infants whose mothers had the HFE variant. The interaction was not present between the HFE variants and maternal blood Pb or cord blood Pb concentrations.

5.3.3.5 Other Genetic Polymorphisms

Some other genetic polymorphisms were also examined as to whether they modify Pb-related health effects, but only limited data were available for these polymorphisms. These include dopamine receptor D4 (DRD4), dopamine receptor D2 (DRD2), dopamine transporter (DAT1), glutathione S-transferase Mu 1 (GSTM1), tumor necrosis factor-*alpha* (TNF-α), endothelial nitric oxide synthase (eNOS), and various SNPS.

A prospective birth cohort reported that increasing blood Pb levels were associated with poorer rule learning and reversal, spatial span, and planning in their study population (Froehlich et al., 2007). These inverse associations were exacerbated among those

lacking DRD4-7. A study of prenatal and postnatal Pb levels in Mexico City reported no modification of the associations between Pb levels and neurocognitive development by DRD2 or DAT1 (Kordas et al., 2011).

A study of university students in South Korea reported blood Pb levels to be associated with biomarkers of inflammation among individuals with GSTM1 null genotype and not among individuals with GSTM1 present (Kim et al., 2007). This study of blood Pb levels and inflammation also examined individuals with TNF-α GG, GA, or AA alleles. An association was present for those with TNF-α GG but not for those with TNF-α GA or AA.

A study of blood Pb and plasma NO_X reported no overall association but did report an inverse correlation among subjects with the eNOS TC+CC genotype (Barbosa et al., 2006c). No correlation was observed for subjects with the eNOS TT genotype; however the number of subjects in this group was small, especially for those with high blood Pb levels.

One study examined how the association between occupational Pb exposure and brain tumors varied among multiple single nucleotide polymorphisms (SNPs) (Bhatti et al., 2009). No effect measure modification of the association between Pb and glioma was observed for any of the SNPs. GPX1 (the gene encoding for glutathione peroxidase 1) modified the association for glioblastoma multiforme and meningioma. The association between Pb and glioblastoma multiforme was also modified by a RAC2 (the gene encoding for Rac2) variant, and the association between Pb and meningioma was also modified by XDH (the gene encoding for xanthine dehydrogenase) variant.

Overall, studies of ALAD observed increased Pb-related health effects associated with certain gene variants. Other genes, such as VDR, HFE, DRD4, GSTM1, TNF-α, and eNOS, may also affect the risk of Pb-related health effects but conclusions are limited due to the small number of studies.

5.3.4 Pre-existing Diseases/Conditions

Studies have also been performed to examine whether certain morbidities increase an individual's risk of Pb-related effects on health. Recent studies have explored relationships for autism, diabetes, and hypertension. In general, consistent associations are observed indicating that hypertension increases the risk of Pb-related renal effects but the evidence is limited for other pre-existing conditions. Although there are many childhood conditions that could hypothetically increase the risk of Pb-related health effects in children (e.g. prenatal exposure to alcohol or drugs, birth asphyxia, head trauma, sickle cell anemia) epidemiologic studies to evaluate such hypotheses are unavailable and therefore they are not discussed.

5.3.4.1 Autism

Rates of individuals with autism have increased in recent years. A study reported a prevalence rate in 2006 of 9.0 per 1,000 individuals (95% CI: 8.6, 9.3) determined from a monitoring network (Autism and Developmental Disabilities Monitoring Network) with 11 sites across the U.S. (CDC, 2009).

A cross-sectional study of children with and without autism examined the association between blood Pb levels and various immune function and inflammation genes (Tian et al., 2011). Blood Pb levels of children with and without autism were associated with expression of the genes under study; however, the associations observed were in opposite directions (for children with autism, increased blood Pb levels were associated with increased expression, whereas for children without autism, increased blood Pb levels were associated with decreased expression).

5.3.4.2 Diabetes

Approximately 8% of U.S. adults have diabetes (Pleis et al., 2009). A few studies have been conducted to investigate the possibility of diabetes as a modifying factor for Pb and various health outcomes.

Differences in the association between bone and blood Pb levels and renal function for individuals with and without diabetes at baseline were examined using the Normative Aging Study cohort (Tsaih et al., 2004). Tibia and blood Pb levels were positively associated with measures of poor renal function among individuals with diabetes but not among individuals without diabetes. However, this association was no longer statistically significant after the exclusion of individuals who were hypertensive or who used diuretic

medications. Another study with this cohort reported no associations between bone Pb and heart rate variability, which did not differ among those with and without diabetes (Park et al., 2006).

The NHANES III data were used to evaluate whether the association between blood Pb and both all-cause and cardiovascular mortality varied among individuals with and without diabetes (Menke et al., 2006). The 95% CIs among those with diabetes were large and no difference was apparent among those with and without diabetes.

Overall, recent epidemiologic studies found that associations between Pb concentrations and health outcomes did not differ for individuals with and without diabetes. However, results from the 2006 Pb AQCD found that individuals with diabetes are at "increased risk of Pb-associated declines in renal function" (U.S. EPA, 2006b). Future research examining associations between Pb and renal function, as well as other health outcomes, among individuals with and without diabetes will inform further on the potential for increased risk among individuals with diabetes.

5.3.4.3 Hypertension

Hypertension affects approximately 24% of adults in the U.S. and the prevalence of hypertension increases with age (61% of individuals ≥ 75 years old have hypertension) (Pleis et al., 2009).

The Normative Aging Study mentioned above evaluating modification of the association between Pb levels and renal function by diabetes also examined modification by hypertensive status (Tsaih et al., 2004). The association between tibia Pb and renal function, measured by change in serum creatinine, was present among individuals with hypertension but not among individuals that were normotensive. Models of the follow-up serum creatinine levels demonstrated an association with blood Pb for individuals with hypertension but not individuals without hypertension (this association was not present when using tibia or patella Pb). Another study using this population examined modification of the association between bone Pb and heart rate variability, measured by low frequency power, high frequency power, and their ratio (Park et al., 2006). Although a statistically significant association between bone Pb and heart rate variability was not observed among individuals with or without hypertension, the estimates were different, with greater odds for individuals with hypertension (bone Pb levels were positively related to low frequency power and the ratio of low frequency to high frequency power and were inversely related to high frequency power).

A study using the NHANES III cohort reported a positive association between blood Pb levels and both all-cause and cardiovascular mortality for individuals with and without hypertension but the associations did not differ based on hypertensive status (Menke et al., 2006).

The 2006 Pb AQCD reported that individuals with hypertension had increased risk of Pb-related effects on renal function (U.S. EPA, 2006b). This is supported by recent epidemiologic studies. As described above, studies of Pb-related effects on renal function and heart rate variability have observed some differences among individuals with hypertension, but the difference between adults with and without hypertension was not observed for Pb-related mortality.

Overall, studies of Pb-related health effects related to pre-existing conditions have some evidence of a potential increased risk of Pb-related health effects. The evidence is consistent for Pb-related renal effects and hypertension but is limited for other pre-existing conditions.

5.3.5 Smoking Status

The rate of smoking among adults 18 years and older in the U.S. is approximately 20% and about 21% of individuals identify as former smokers (Pleis et al., 2009). Studies of Pb and various health effects have examined smoking as an effect measure modifier and reported inconsistent results.

A study of blood Pb levels and all-cause and cardiovascular mortality reported no modification of this association by smoking status, measured as current, former, or never smokers (Menke et al., 2006). The Normative Aging Study also examined the association between blood and bone Pb levels and renal function and also reported no interaction with smoking status (Tsaih et al., 2004).

A study of Pb-exposed workers and controls reported similar levels of absolute neutrophil counts (ANC) across Pb exposure categories among nonsmokers (Di Lorenzo et al., 2006). However, among current smokers, higher Pb exposure was associated with higher ANC. Additionally, a positive relationship was observed between higher blood Pb levels and TNF-α and granulocyte colony-stimulating factor (G-CSF) among both smokers and nonsmokers, but this association was greater among smokers (Di Lorenzo et al., 2007). A recent study of fertile and infertile men examined blood and seminal plasma Pb levels for smokers and nonsmokers (Kiziler et al., 2007). The blood and seminal plasma Pb levels were higher for smokers of both fertile and infertile groups. Additionally, the Pb levels were lowest among nonsmoking fertile men and highest among smoking infertile men.

Maternal smoking during pregnancy was examined in a study of children's concurrent blood Pb levels and prevalence of attention-deficit/hyperactivity disorder (ADHD) among children aged 8-15 years. An interaction was observed between children's current blood Pb levels and prenatal tobacco smoke exposure; those children with high Pb levels and prenatal tobacco smoke exposure had the highest odds of ADHD (Froehlich et al., 2009).

Overall, the studies have inconsistent findings on whether smoking modifies the relationship between Pb levels and health effects. Future studies of Pb-related health effects and current, former, second-hand, and prenatal smoking exposures among various health endpoints will aid in determining changes in risk by this factor.

5.3.6 Socioeconomic Status

According to the Current Population Survey Annual Social and Economic Supplements conducted by the Census Bureau, in 2011 15.0% of Americans lived in poverty (average poverty threshold for family of 4 was $23,021) (DeNavas-Walt et al., 2012). Few studies have compared blood Pb level effect estimates among groups in different sociodemographic strata. Larger blood Pb-associated decreases in cognitive function were found with lower SES in some studies (Ris et al., 2004; Tong et al., 2000; Bellinger et al., 1990). In contrast, a meta-analysis of eight studies found a smaller decrement in Full Scale Intelligence Quotient (FSIQ) for studies in disadvantaged populations than for studies in advantaged populations (Schwartz, 1994). While results indicate that blood Pb level is associated with FSIQ deficits in both higher and lower sociodemographic groups, they do not clearly indicate whether groups with different socioeconomic status differ in Pb-related changes for cognitive function.

5.3.7 Race/Ethnicity

Based on the 2010 Census, 63.7% of the U.S. population is comprised of non-Hispanic whites. Approximately 12.2% of people reported their race/ethnicity as non-Hispanic black and 16.3% reported being Hispanic (Humes et al., 2011). Studies of multiple Pb-related health outcomes examined effect measure modification by race/ethnicity and although there are a limited number of studies, there is evidence that certain Pb-related outcomes may vary by race/ethnicity.

A study of adults from the NHANES III cohort examined the association between blood Pb levels and all-cause and cardiovascular mortality (Menke et al., 2006). Stratified analyses were conducted for non-Hispanic whites, non-Hispanic blacks, and Mexican

Americans and no interaction for race/ethnicity was reported. Other studies have also used NHANES cohorts to study blood Pb levels and hypertension (Scinicariello et al., 2010; Muntner et al., 2005). While no association was observed between blood Pb and hypertension for non-Hispanic whites or Hispanics, a positive association was reported for non-Hispanic blacks in a study using the NHANES III cohort (Scinicariello et al., 2010). In another study, although none of the associations between blood Pb levels and hypertension were statistically significant, increased odds were observed among non-Hispanic blacks and Mexican Americans but not for non-Hispanic whites (Muntner et al., 2005).

A study of girls aged 8-18 years from the NHANES III cohort reported an inverse association between blood Pb levels and pubertal development among blacks and Mexican Americans (Selevan et al., 2003). For non-Hispanic whites, the associations were in the same direction but did not reach statistical significance. Of note, less than 3% of non-Hispanic whites had blood Pb levels over 5 µg/dL, whereas 11.6% and 12.8% of blacks and Mexican Americans, respectively, had blood Pb levels greater than 5 µg/dL.

A study linking educational testing data for 4th grade students in North Carolina reported declines in reading and mathematics scores with increasing levels of blood Pb (Miranda et al., 2007a). Although not quantitatively reported, a figure in the study depicted the association stratified by race, and the slopes appeared to be similar for white and black children.

Blood Pb and asthma incidence was examined for white and black children living in Michigan (Joseph et al., 2005). When utilizing separate referent groups for the two races, the only association is an increase among whites (although not statistically significant), but when restricting to the highest blood Pb levels, the association was no longer apparent. Whites with low blood Pb levels were used as the referent group for both races in additional analysis. Although the estimates were elevated for black children compared to white children (including at the lowest blood Pb levels), the confidence intervals for the associations overlapped indicating a lack of a difference by race.

The results of these recent epidemiologic studies provide some evidence that there may be race/ethnicity-related increased risk with higher Pb levels for certain outcomes, although the overall understanding of potential effect measure modification by race/ethnicity is limited by the small number of studies. Additionally, these results may be confounded by other factors, such as socioeconomic status or nutritional factors.

5.3.8 Body Mass Index

In the U.S. self-reported rates of obesity were 26.7% in 2009, up from 19.8% in 2000 (Sherry et al., 2010). The NHANES III cohort was utilized in a study of blood Pb levels and all-cause and cardiovascular mortality, which included assessment of the associations by obesity (Menke et al., 2006). Positive associations were observed among individuals within both categories of body mass index (BMI; normal [<25 kg/m^2] and overweight/obese [≥ 25 kg/m^2], determined using measured values of height and weight) but there was no difference in the association between the two categories. Using the Normative Aging Study data, an investigation of bone Pb levels and heart rate variability was performed and reported slight changes in the association based on the presence of metabolic syndrome; however, none of the changes resulted in associations that were statistically significant (Park et al., 2006). Overall, no modification by BMI or obesity was observed among recent epidemiologic studies, but the available epidemiologic and supporting toxicological studies are limited.

5.3.9 Alcohol Consumption

There are a limited number of studies examining alcohol as a factor affecting Pb-related risk. A study using the Normative Aging Study cohort investigated whether the association between blood and bone Pb levels and renal function would be modified by an individual's alcohol consumption (Tsaih et al., 2004). No interaction with alcohol consumption was observed. However, a toxicological study reported that ethanol potentiated the effect of Pb exposure by decreasing renal total protein sulfhydryls (endogenous antioxidants) in rats. Pb and ethanol also decreased other endogenous renal antioxidants (glutathione and non-protein sulfhydryls) (Jurczuk et al., 2006). Overall, evidence to determine if alcohol consumption is a potential at-risk factor is of limited quantity and consistency.

5.3.10 Nutritional Factors

Different components of diet may affect the association between Pb concentrations and health outcomes. Recent epidemiologic and toxicological studies of specific mineral intakes/dietary components are detailed below but evidence is limited.

5.3.10.1 Calcium

Using the Normative Aging Study (NAS) cohort, researchers examined the association between Pb levels and hypertension, modified by calcium intake over the year before Pb measurement (determined by a food frequency questionnaire) (Elmarsafawy et al., 2006). The associations between Pb levels (measured and modeled separately for blood, patella, and tibia) and hypertension did not differ based on dichotomized calcium intake (800 mg/day).

5.3.10.2 Iron

The 2006 Pb AQCD included studies that indicated individuals with Fe deficiency and malnourishment had greater inverse associations between Pb and cognition (U.S. EPA, 2006b). A recent epidemiologic study of pubertal development among girls observed inverse associations between blood Pb and inhibin B. This association was modified by Fe deficiency; girls with Fe deficiency had a stronger inverse association between Pb and inhibin B than those who were Fe sufficient (Gollenberg et al., 2010). Toxicological studies also reported that Fe-deficient diets exacerbate or potentiate the effect of Pb. A study of pregnant rats given an Fe-deficient diet and exposed to Pb through drinking water over GD6-GD14, had decreased litter size, more pups with reduced fetal weight and reduced crown-rump length, increased litter resorption, and a higher dam blood Pb level in the highest exposure groups (Singh et al., 1993b; Saxena et al., 1991). Thus, in this model, Fe deficiency makes rat dams more at risk for Pb-dependent embryo and fetotoxicity (Singh et al., 1993b).

5.3.10.3 Folate

A study by Kordas et al. (2009) examined Pb levels and birth size among term births in Mexico City. The authors reported no interaction between maternal tibia Pb and folate levels.

5.3.10.4 Protein

No recent epidemiologic studies have evaluated protein intake as a factor affecting Pb-related health effects. However, a toxicological study demonstrated that differences in maternal protein intake levels could affect the extent of Pb-induced immunotoxicity among offspring (Chen et al., 2004).

In sum, the evidence is limited for most dietary factors but evidence for Fe deficiency as a factor that potentially increases risk of Pb-induced effects is present and coherent in epidemiologic and toxicological studies.

5.3.11 Stress

Recent studies have examined modification of the association between Pb and various health effects by stress-level. Evidence demonstrates that higher stress levels may increase the risk of Pb-related health effects.

A study of bone (tibia and patella) Pb levels and hypertension reported modification of the association by perceived stress levels (Peters et al., 2007). Among individuals with greater perceived stress levels, stronger associations between blood Pb levels and hypertension were present. Among the same study population, higher perceived stress was also reported to affect the association between blood Pb levels and cognitive function; the higher stress group showed a greater inverse association between Pb and cognitive function than those in the low stress group (Peters et al., 2008). In another study, the inverse association between tibia Pb levels and some measures of cognitive function were similarly strengthened by neighborhood psychosocial hazards (Glass et al., 2009).

Toxicological studies have demonstrated that early life exposure to Pb and maternal stress can result in toxicity related to multiple systems (Rossi-George et al., 2009; Cory-Slechta et al., 2008; Virgolini et al., 2008a; Virgolini et al., 2008b), including dysfunctional corticosterone responses (Rossi-George et al., 2009; Virgolini et al., 2008b). Additionally, toxicological studies have demonstrated that stressors to the immune system can also affect associations with Pb exposure. Chickens with low Pb exposure in ovo, with additional viral stressors, had increased immune cell mobilization and trafficking dysfunction (Lee et al., 2002). Similarly, mice with neonatal Pb exposure, and an additional immune challenge, had a sickness behavior phenotype, likely driven by IL-6 production (Dyatlov and Lawrence, 2002).

Although examined in a small number of studies, which limited the power to provide coherence, recent epidemiologic studies observed modification of the association between Pb and various nervous system health effects by stress-level. Increased risk of Pb-related health effects by stress is further supported by toxicological studies.

5.3.12 Maternal Self-Esteem

Maternal self-esteem has been shown to modify associations between blood Pb levels and health effects in children. Surkan et al. (2008) studied the association between children's blood Pb levels and Bayley's MDI and Psychomotor Development Index (PDI) among mother-child pairs. High maternal self-esteem was independently associated with higher MDI score and also appeared to attenuate the negative effects of the child's increased blood Pb levels on MDI and PDI scores. Greater decreases in MDI and PDI were associated with increased blood Pb levels among children whose mothers were in the lower quartiles of self-esteem.

5.3.13 Cognitive Reserve

Cognitive reserve has been defined as "the maintenance of cognitive performance in spite of ongoing underlying brain pathology" (Bleecker et al., 2007a). A study of Pb smelter workers reported that an inverse association between lifetime weighted blood Pb levels and cognitive function was present among workers with low cognitive reserve (measured using a reading achievement test) but no association was present in workers with high cognitive reserve (Bleecker et al., 2007a). Inverse associations between lifetime-weighted blood Pb levels and motor functions existed among all workers regardless of cognitive reserve. No other recent epidemiologic studies were performed examining cognitive reserve as a factor affecting risk of Pb-related health outcomes, thus providing limited evidence to conclude that cognitive reserve is a potential at-risk factor.

5.3.14 Other Metal Exposure

The 2006 Pb AQCD reported that the majority of studies that examined other toxicants did so as confounders and not as effect measure modifiers (U.S. EPA, 2006b). Recent epidemiologic studies have begun to explore the possible interaction between Pb exposure and co-exposures with other metals and reported increased risk of Pb-related outcomes. These studies, as well as toxicological studies of these metals, are described below.

5.3.14.1 Cadmium

In a study of girls in the NHANES III cohort, inverse associations were observed between blood Pb and inhibin B concentrations (Gollenberg et al., 2010). These inverse

associations were stronger among girls with high cadmium (Cd) and high Pb compared to those with high Pb and low Cd. Additionally, higher blood Pb and Cd levels together were positively associated with albuminuria and reduced estimated glomerular filtration rate, compared to those with the lowest levels of Pb and Cd (Navas-Acien et al., 2009).

Toxicological studies reported that in rats, the addition of Cd to Pb exposure reduced the histological signs of renal toxicity from each element alone; however, urinary excretion of porphyrins were increased, indicating that although measured tissue burdens of Pb were reduced, the biologically available fraction of Pb was actually increased (Wang and Fowler, 2008). In other studies, Cd synergistically exacerbated Pb-dependent renal mitochondrial dysfunction (Wang et al., 2009c).

Overall, epidemiologic and toxicological studies have reported increased risk of Pb-related health effects among those with high Cd levels as well; however, the number of studies examining both metals is small.

5.3.14.2 Manganese

Among children in South Korea taking part in a study of IQ, an interaction was reported between Pb and manganese (Mn) blood levels (Kim et al., 2009b). Children with high blood Mn levels were observed to have reductions in full scale IQ and verbal IQ associated with increased blood Pb levels, whereas no association between blood Pb levels and full scale IQ and verbal IQ were noted among those children with low blood Mn levels. No effect measure modification by Mn was observed for the association between blood Pb levels and performance IQ. A study performed among children in Mexico City observed greater decreases in neurodevelopment with increases in blood levels of Pb and Mn at 12 months, compared to decreases in neurodevelopment observed for increased Pb levels with low levels of Mn (Claus Henn et al., 2012). No interaction was observed between the two metals and neurodevelopment at 24 months.

Overall, studies have reported increased risk of various health effects with exposure to other metals in addition to Pb; however, this is limited by the small number of studies. Toxicological studies, when available, have provided support for these findings.

5.4 Summary

Table 5-5 provides an overview of the factors examined as potentially increasing the risk of Pb-related health effects based on the recent evidence integrated across disciplines. They are classified according to the criteria discussed in the introduction to this chapter.

Table 5-5 Summary of evidence for factors that potentially increase the risk of Pb-related health effects.

Factor Evaluated	Classification
Childhood (Sections 5.2.1, 5.3.1)	Adequate
Older Adulthood (Sections 5.2.1, 5.3.1)	Suggestive
Sex (Sections 5.2.2, 5.3.2)	Suggestive
Genetics (Section 5.3.3)	Suggestive
Pre-existing Disease[a] (Section 5.3.4)	Suggestive
Smoking Status (Section 5.3.5)	Inadequate
Socioeconomic Status (SES) (Sections 5.2.4, 5.3.6)	Suggestive
Race/Ethnicity (Sections 5.2.3, 5.3.7)	Adequate
Proximity to Pb Sources (Section 5.2.5)	Adequate
Residential Factors (Section 5.2.6)	Adequate
Body Mass Index (BMI) (Section 5.3.8)	Inadequate
Alcohol Consumption (Section 5.3.9)	Inadequate
Nutrition (Section 5.3.10)	Adequate
Stress (Section 5.3.11)	Suggestive
Maternal Self-Esteem (Section 5.3.12)	Inadequate
Cognitive Reserve[a] (Section 5.3.13)	Inadequate
Other Metals (Section 5.3.14)	Suggestive

[a]Possible mediator

There are consistent findings, coherent across disciplines that adequate evidence exists to conclude that childhood is an at-risk lifestage. Among children, the youngest age groups were observed to be most at risk of elevated blood Pb levels, with levels decreasing with increasing age of the children. Children may have increased exposure to Pb compared with adults because children's behaviors and activities (including increased hand-to-mouth contact, crawling, and poor hand-washing), differences in diets, and biokinetic factors.

For adults, elevated Pb biomarkers were associated with increasing age. It is generally thought that these elevated levels are related to remobilization of stored Pb during bone loss and/or higher historical Pb exposures. Studies of older adults had inconsistent findings for effect measure modification of Pb-related mortality but no difference was observed for other health effects. However, toxicological studies support the possibility of age-related differences in Pb-related health effects. The overall evidence is suggestive that older adults are a potential at-risk population based on limited epidemiologic evidence but support from toxicological studies and differential exposure studies.

Some studies suggest that males at some ages have higher blood Pb levels than comparably aged females; this was supported by stratifying the total sample of NHANES subjects. Sex-based differences appeared to be prominent among the adolescent and adult age groups but were not observed among the youngest age groups (1-5 years and 6-11 years). Studies of effect measure modification of Pb and various health endpoints by sex were inconsistent; although it appears that there are some differences in associations for males and females. This is also observed in toxicological studies. Overall, there is suggestive evidence to conclude that sex is a potential at-risk factor, limited due to inconsistencies between whether males or females are at greater risk of certain outcomes.

Regarding race and ethnicity, recent data suggest that the difference in blood Pb levels between black and white subjects is decreasing over time, but black subjects still tend to have higher Pb body burden and Pb exposures than white subjects. Compared to whites, non-white populations were observed to be more at risk of Pb-related health effects (i.e. hypertension, delayed puberty onset); however, this could be related to confounding by factors such as SES or differential exposure levels, which was noted in some of the epidemiologic studies. Studies of race/ethnicity provide adequate evidence that race/ethnicity is an at-risk factor based on the higher exposure observed among non-white populations and some modification observed in studies of associations between Pb levels and health effects.

Similar to race and ethnicity, the gap between SES groups with respect to Pb body burden appears to be diminishing. Studies of SES and its relationship with Pb-related health effects are limited and different studies demonstrate increased risk among higher or lower SES groups, providing limited evidence to determine if SES is an at-risk factor for Pb-related health effects. However, biomarkers of Pb exposure have been shown to be higher among lower SES groups even in recent studies in which differences among SES groups have lessened. Therefore, the evidence is suggestive to conclude that low SES is a potential at-risk factor for Pb-related health effects.

There is evidence associating proximity to areas with Pb sources, including areas with large industrial sources, with increased Pb body burden and risk of Pb exposure. High concentrations of ambient air Pb have been measured near sources, compared with large urban areas without sources. Additionally, high Pb exposures have been documented near Superfund sites.

Studies utilizing the NHANES dataset have reported increased Pb biomarker measures related to increase house dust Pb levels, homes built after 1950, and renovation of pre-1978 homes. These findings were consistent with those of several high quality studies. Thus, there is adequate evidence that residing in a residence with Pb exposures will increase the risk of Pb-related health effects.

There is suggestive evidence to conclude that various genes are potentially modifying the associations between Pb and health effects. Epidemiologic and toxicological studies reported that ALAD variants may increase the risk of Pb-related health effects. Other genes examined that may also affect risk of Pb-related health effects were VDR, DRD4, GSTM1, TNF-α, eNOS, and HFE, although the number of studies examining effect measure modification by these genes was small.

Among nutritional factors, diets sufficient in minerals such as Ca^{2+}, Fe, and Zn offer some protection from Pb exposure by preventing or competing with Pb for absorption in the GI tract. Additionally, those with Fe deficiencies were observed to be an at-risk population for Pb-related health effects in both epidemiologic and toxicological studies. Thus, there is adequate evidence across disciplines that some nutritional factors contribute to a population being at increased risk. Other nutritional factors, such as Ca^{2+}, Zn, and protein intake, demonstrated the potential to modify associations between Pb and health effects in toxicological studies.

There was suggestive evidence for several other factors as potentially increasing the risk of Pb-related health effects: pre-existing diseases/conditions, stress, and co-exposure with other metals. Pre-existing diseases/conditions have the potential to affect the risk of Pb-related health effects. Recent epidemiologic studies did not support modification of associations between Pb and health endpoints by the prevalence of diabetes; however, past studies have found individuals with diabetes to be an at-risk population with regard to renal function. Hypertension was observed to be a factor affecting risk in both past and recent epidemiologic studies. Studies of Pb levels and both renal effects and heart rate variability demonstrated greater odds of the associations among hypertensive individuals compared to those that are normotensive. Epidemiologic studies also examined autism as potential factors affecting Pb-related health effects; differences were observed but few studies were available to examine this factor. Stress was evaluated as a factor that potentially increases the risk of Pb-related health outcomes and although limited by the small number of epidemiologic studies, increased stress was observed to impact the association between Pb and health endpoints. Toxicological studies supported this finding. Finally, interactions between Pb and co-exposure with other metals were evaluated in recent epidemiologic and toxicological studies of health effects. High levels of other metals, such as Cd and Mn, were observed to result in greater effects for the associations between Pb and various health endpoints but evidence was limited due to the small number of studies.

Finally, there was inadequate evidence to conclude that smoking, BMI, alcohol consumption, maternal self-esteem, and cognitive reserve are potential at-risk factors due to limited quantities of studies regarding their effect on Pb-related health outcomes.

Epidemiologic studies examining smoking as a factor potentially affecting risk reported mixed findings. It is possible that smoking modifies the effects of only some Pb-related health outcomes. In the limited number of studies, modification of associations between Pb and various health effects (mortality and heart rate variability) was not observed for BMI/obesity. Also, no modification was observed in an epidemiologic study of renal function examining alcohol consumption as a modifier, but a toxicological study supported the potential of alcohol to affect risk. Maternal self-esteem was examined in an epidemiologic study and individuals with mothers who had lower self-esteem had greater Pb-related decreases in MDI and PDI. An epidemiologic study evaluated cognitive reserve as a modifier of the associations between Pb and cognitive and motor functions. Cognitive reserve was an effect measure modifier for the association between Pb and cognitive function but not motor function.

Common metrics that would allow estimation of the magnitude of increased risk, conferred by the at- risk factors considered in this chapter, are generally unavailable. There is variation in the Pb measures used (e.g. blood or bone Pb) and in population groups (e.g. different age groupings for children). In addition, available studies show differences in magnitudes of association that vary within and across each outcome examined for an at-risk factor. For example, studies of race report no effect measure modification by race for some outcomes (mortality, asthma, and scores on reading and math examinations) (Miranda et al., 2007a; Menke et al., 2006; Joseph et al., 2005), whereas other outcomes (delayed puberty onset and hypertension) demonstrated effect measure modification (Scinicariello et al., 2010; Muntner et al., 2005; Selevan et al., 2003). In sum, there is adequate evidence that several factors – childhood, race/ethnicity, nutrition, residential factors, and proximity to Pb sources – confer increased risk of Pb-related health effects. However, the evidence is not conducive to quantifying the differences in magnitude of increased risk related to these factors.

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CHAPTER 6 ECOLOGICAL EFFECTS OF LEAD

This chapter synthesizes and evaluates the most policy-relevant science to help form the foundation for the review of the secondary (welfare-based) NAAQS for Pb. The Clean Air Act definition of welfare effects includes, but is not limited to, effects on soils, water, wildlife, vegetation, visibility, weather, and climate, as well as effects on materials, economic values, and personal comfort and well-being. This chapter discusses the effects of Pb on ecosystem components and processes and is organized into five sections. The introduction (Section 6.1) presents the organizing principles of this chapter and several important general ecology concepts. An overview of fate and transport of Pb in ecosystems including measured concentrations of this metal in various environmental media (i.e., soil, water, sediment) is presented in Section 6.2. Section 6.3 reviews the effects of Pb on terrestrial ecosystems; how soil biogeochemistry affects Pb bioavailability, biological effects of Pb exposure and subsequent vulnerability of particular ecosystems. A similar discussion of the effects of Pb on freshwater and saltwater ecosystems is presented in Section 6.4, including water-only exposures and sediment-related effects. The terrestrial, freshwater and saltwater sections each conclude with an integrative synthesis of new evidence for Pb effects and causal determinations, based on the synthesis of new evidence and findings from previous Pb AQCDs. Section 6.5 summarizes the causal determinations. Areas not addressed here include literature related to ingestion of Pb shot or pellets and studies that examine human health-related endpoints which are described in other chapters of this document.

6.1 Introduction to Ecological Concepts

Metals, including Pb, occur naturally in the environment at measurable concentrations in soils, sediments, and water. Organisms have developed adaptive mechanisms for living with metals, some of which are required micronutrients (but not Pb). However, anthropogenic enrichment can result in concentrations that exceed the capacity of organisms to regulate internal concentrations, causing a toxic response and potentially death. Differences in environmental chemistry may enhance or inhibit uptake of metal from the environment, thus creating a spatial patchwork of environments that are at greater risk than other environments. Similarly, organisms vary in their degree of adaptation to, or tolerance of, the presence of metals. These fundamental principles of how metals interact with organisms and ecosystems are described in detail in EPA's *Framework for Metals Risk Assessment* (U.S. EPA, 2007c). This section introduces critical concepts for understanding how Pb from atmospheric deposition may affect

organisms, communities, and ecosystems. The sections that follow provide more detail for how aquatic and terrestrial ecosystems respond to Pb and how environmental chemistry interacts with organisms to affect exposure and uptake.

6.1.1 Ecosystem Scale, Function, and Structure

For this assessment, an ecosystem is defined as the interactive system formed from all living organisms (biota) and their abiotic (chemical and physical) environment within a given area (IPCC, 2007).The boundaries of what could be called an ecosystem are somewhat arbitrary, depending on the focus of interest or study. Thus, the extent of an ecosystem may range from very small spatial scales to, ultimately, the entire Earth (IPCC, 2007). Ecosystems cover a hierarchy of spatial scales and can comprise the entire globe, biomes at the continental scale, or small, well-circumscribed systems such as a small pond (U.S. EPA, 2008e). A pond may be a small but complex system with multiple trophic levels ranging from phytoplankton to several feeding guilds of fish plus fish-eating birds or mammals. A large lake, on the other hand, may be a very simple ecosystem, such as the Great Salt Lake in Utah that covers approximately 1,700 square miles but contains only bacteria, algae, diatoms, and two invertebrate species. All ecosystems, regardless of size or complexity, share the commonality of multiple interactions between biota and abiotic factors, and a reduction in entropy through energy flow from photosynthetic organisms to top predators. This includes both structural (e.g., soil type and food web trophic levels) and functional (e.g., energy flow, decomposition, nitrification) attributes. Changes are often considered undesirable if important structural or functional components of ecosystems are altered following pollutant exposure (U.S. EPA, 1998).

Ecosystems are most often defined by their structure, and are based on the number and type of species present. Structure may refer to a variety of measurements including the species richness, abundance, community composition, and biodiversity as well as landscape attributes. Individual organisms of the same species are similar in appearance and genetics, and can interbreed and produce fertile offspring. Interbreeding groups of individual organisms within the same species that occupy some defined geographic space form populations, and populations of different species form communities (Barnthouse et al., 2008). The community composition may also define an ecosystem type, such as a pine forest or a tall grass prairie. Pollutants can affect the ecosystem structure at any of these levels of biological organization (Suter et al., 2005). Individual plants or animals may exhibit changes in metabolism, enzyme activities, hormone function, or overall growth rates or may suffer gross lesions, tumors, deformities, or other pathologies. Effects on the nervous system of animals may cause behavioral changes that alter

breeding behaviors or predator avoidance. However, only some organism-level endpoints such as growth, survival, and reproductive output have been definitively linked to effects at the population level and above. Examples of organism-level endpoints with direct links to population level effects include mass mortality, gross anomalies, survival, fecundity and growth (Suter et al., 2004). Population level effects of pollutants include changes over time in abundance or density (number of individuals in a defined area), age or sex structure, and production or sustainable rates of harvest (Barnthouse et al., 2008). Community level attributes affected by pollutants include species richness and abundance (also known as biodiversity), dominance of one species over another, or size (area) of the community. Pollutants may affect communities in ways that are not observable in organisms or populations (Bartell, 2007), including: (1) effects resulting from interactions between species, such as altering predation rates or competitive advantage; (2) indirect effects, such as reducing or removing one species from the assemblage and allowing another to emerge (Petraitis and Latham, 1999); and (3) alterations in trophic structure.

Alternatively, ecosystems may be defined on a functional basis. “Function” refers to the suite of processes and interactions among the ecosystem components and their environment that involve nutrient and energy flow as well as other attributes including water dynamics and the flux of trace gases such as rates of photosynthesis, decomposition, nitrification, or carbon cycling. Pollutants may affect abiotic conditions (e.g., soil chemistry), which indirectly influences biotic structure and function (Bartell, 2007). Feedback loops or networks influence the stability of the system, and can be mathematically described through simplistic or complex process, or energy flow, models (Bartell, 2007). For example, the Comprehensive Aquatic Systems Model (CASM) is a bioenergetics-based multi-compartment model that describes the daily production of biomass (carbon) by populations of aquatic plants and animals over an annual cycle (DeAngelis et al., 1989). CASM, originally designed to examine theoretical relationships between food web structure, nutrient cycling, and ecosystem stability, has since been adapted for risk assessments and has been applied to numerous lakes with a variety of pollutants (Bartell, 2007). Likewise, other theoretical ecosystem models are being modified for use in assessing ecological risks from pollutant exposures (Bartell, 2007).

Some ecosystems, and some aspects of particular ecosystems, are less vulnerable to long-term consequences of pollutant exposure. Other ecosystems may be profoundly altered if a single attribute is affected. Thus, spatial and temporal definitions of ecosystem structure and function become an essential factor in defining impacted ecosystem services and critical loads of particular pollutants, either as single pollutants or in combination with other stressors. Both ecosystem services (Section 6.1.2) and critical loads (Section 6.1.3) serve as benchmarks or measures of the impacts of pollutants on ecosystems.

6.1.2 Ecosystem Services

Ecosystem structure and function may be translated into ecosystem services (Daily, 1997). Ecosystem services are the benefits people obtain from ecosystems (UNEP, 2003). Ecosystem services are defined as the varied and numerous ways that ecosystems are important to human welfare and how they provide many goods and services that are of vital importance for the functioning of the biosphere. This concept has gained recent interest and support because it recognizes that ecosystems are valuable to humans, and are important in ways that are not generally appreciated (Daily, 1997). Ecosystem services also provide a context for assessing the collective effects of human actions on a broad range of the goods and services upon which humans rely.

In general, both ecosystem structure and function play essential roles in providing goods and services. Ecosystem processes provide diverse benefits including absorption and breakdown of pollutants, cycling of nutrients, binding of soil, degradation of organic waste, maintenance of a balance of gases in the air, regulation of radiation balance and climate, and fixation of solar energy (WRI, 2000; Daily, 1997; Westman, 1977). These ecological benefits, in turn, provide economic benefits and values to society (Costanza et al., 1997; Pimentel et al., 1997). Goods such as food crops, timber, livestock, fish and clean drinking water have market value. The values of ecosystem services such as flood control, wildlife habitat, cycling of nutrients and removal of air pollutants are more difficult to measure (Goulder and Kennedy, 1997).

Particular concern has developed within the past decade regarding the consequences of decreasing biological diversity (Tilman, 2000; Ayensu et al., 1999; Wall, 1999; Chapin et al., 1998; Hooper and Vitousek, 1997). Human activities that decrease biodiversity also alter the complexity and stability of ecosystems and change ecological processes. In response, ecosystem structure and function can be affected (Daily and Ehrlich, 1999; Wall, 1999; Chapin et al., 1998; Levin, 1998; Peterson et al., 1998; Tilman, 1996; Tilman and Downing, 1994; Pimm, 1984). Biodiversity is an important consideration at all levels of biological organization, including species, communities, populations, and ecosystems. Human-induced changes in biotic diversity and alterations in the structure and functioning of ecosystems are two of the most dramatic ecological trends of the past century (U.S. EPA, 2004; Vitousek et al., 1997).

Hassan et al. (2005) identified four broad categories of ecosystem services:

- Supporting services are necessary for the production of all other ecosystem services. Some examples include biomass production, production of atmospheric O_2, soil formation and retention, nutrient cycling, water cycling and provisioning of habitat. Biodiversity is a supporting service in that it is increasingly recognized to sustain many of the goods and services that humans enjoy from ecosystems. These supporting services provide a basis for an additional three higher-level categories of services.
- Provisioning services such as products (Gitay et al., 2001) i.e., food (including game meat, roots, seeds, nuts, and other fruit, spices, fodder), water, fiber (including wood, textiles) and medicinal and cosmetic products.
- Regulating services that are of paramount importance for human society such as (1) carbon sequestration, (2) climate and water regulation, (3) protection from natural hazards such as floods, avalanches, or rock-fall, (4) water and air purification, and (5) disease and pest regulation.
- Cultural services that satisfy human spiritual and aesthetic appreciation of ecosystems and their components.

6.1.3 Critical Loads as an Organizing Principle for Ecological Effects of Atmospheric Deposition

A critical load is defined as, "a quantitative estimate of an exposure to one or more pollutants below which significant harmful effects on specified sensitive elements of the environment do not occur according to present knowledge" (Nilsson and Grennfelt, 1988). Critical loads are a powerful organizing principle for information that links atmospheric deposition with ecological impairment. They allow for heterogeneity in ecosystem sensitivity and exposure which often results in critical load values that vary by ecosystem (e.g., aquatic-water; aquatic-sediment; terrestrial), and differ by endpoint of concern. It is important to consider that critical loads are often calculated assuming steady state conditions (i.e., how much input is required to balance the rate of output), and there may be time required to reach the critical load (i.e., the lag time between onset of exposure and induction of measurable effects). The following types of information are required to calculate a critical load, each of which is discussed in more detail in the subsequent sections of this chapter:

- Ecosystem at risk;
- Receptors of concern (plants, animals, etc.);
- Endpoints of concern (organism, population or community responses, changes in ecosystem services or functions);
- Dose (concentration) - response relationships and threshold levels of effects;
- Bioavailability and bioaccumulation rates;

- Naturally occurring (background) Pb (or other metal) concentrations; and
- Biogeochemical modifiers of exposure.

There is no single "definitive" critical load for a pollutant, partly because critical load estimates reflect the current state-of-knowledge and policy priorities, and also because of local or regional differences among ecosystems (U.S. EPA, 2008e). Changes in scientific understanding may include, for example, expanded information about dose-response relationships, better understanding of bioavailability factors, and improved quantitative models for effects predictions. Changes in policy may include new mandates for resource protection, inclusion of perceived new threats that may exacerbate the effects of the pollutant of concern (e.g., climate change), and a better understanding of the value of ecosystem services.

6.2 Fate and Transport of Pb in Ecosystems

Fate and transport of Pb in ecosystems are difficult to assess because Pb detected in the environment could have multiple sources and passes through various environmental media within a watershed. These issues are described in detail in Section 2.3. Pb can be emitted to air, soil, or water and then cycle through any or all of these media. In addition to primary emission of particle-bound or gaseous Pb to the atmosphere, Pb can be resuspended to the air from soil or dust (Section 6.2.2). Additionally, Pb-bearing PM can be deposited from the air to soil or water through wet and dry deposition. The complicated nature of Pb fate and transport in ecosystems is illustrated in Figure 6-1 in which the Venn diagram depicts how Pb can move through multiple environmental media that encompass both terrestrial and aquatic systems (see also Figure 2-9). The "air/soil/water" arrows illustrate Pb exposures to plants and animals. Many of the studies presented in the subsequent material focus on observations of Pb exposure via one medium: air, soil, sediment, or water.

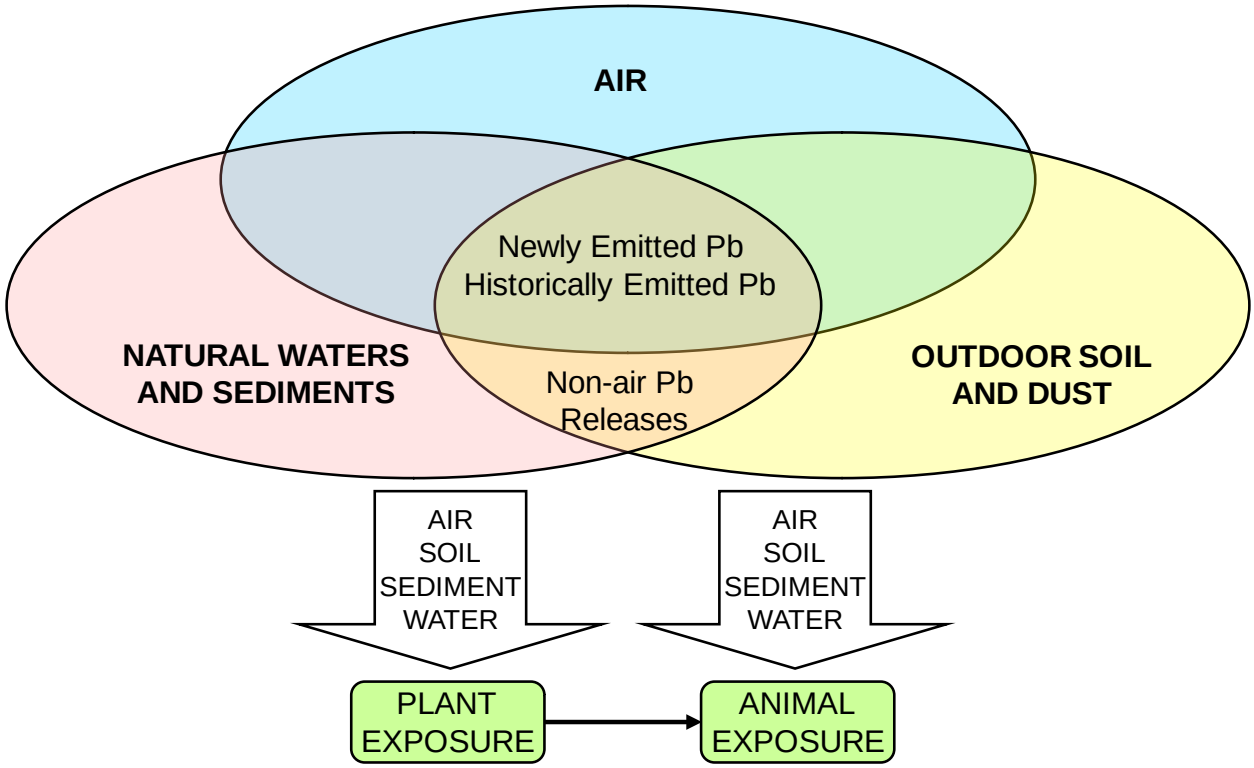


Figure 6-1 Fate of atmospheric Pb in ecosystems.

6.2.1 Fate and Transport

This section provides a brief overview of the fate and transport of Pb in ecosystems. Fate of Pb is determined by the chemical and physical properties of the medium in wet deposition, bodies of water, or soil (e.g., pH, salinity, oxidation status, flow rate and the suspended sediment load and its constituents). Desorption, dissolution, precipitation, sorption and complexation processes can all occur concurrently and continuously, leading to transformations and redistribution of Pb within a watershed. A primary factor controlling fate and transport and the subsequent bioavailability of Pb in both aquatic and terrestrial systems is solubility. In terrestrial systems, Pb is typically bound to organic matter and soil. The most important factors in determining Pb solubility in soils are pH and CEC (Smolders et al., 2009) (Section 6.3.2), while in aquatic systems, solubility varies primarily with pH and water hardness as well as Ca^{2+} concentration, total suspended solids and DOC (Section 6.4.2). Therefore, the pH of water is of primary importance in determining the likely chemical fate of Pb in terms of solubility,

precipitation, or organic complexation. For more detailed information about the fate and transport of Pb, please see Section 2.3.

Soluble Pb in air is mostly removed by wet deposition, and most of the insoluble Pb is removed by dry deposition. As a result, dry deposition is the major removal mechanism for Pb in coarse PM (which is mainly insoluble) and wet deposition is the most important removal mechanism for fine PM and Pb halides (which were more soluble) (Section 2.3.1). Recent research provides considerable evidence that appreciable amounts of Pb can accumulate on coarse PM during transport, and that the physical and chemical characteristics of Pb can be altered by this process due to accompanying transformations (Section 2.3.1.1). Atmospheric removal of metals by wet or dry deposition is largely controlled by solubility of Pb in rain water. The relative importance of wet and dry deposition is highly variable with respect to location and season, probably reflecting both variations in Pb speciation and variations in external factors such as pH and rain water composition (Section 2.3.1.2).

Pb deposited to terrestrial ecosystems may remain in soils or eventually be transported in runoff to streams, lakes or rivers in the watershed. Pb has a relatively long retention time in the organic soil horizon, although its movement through the soil column also suggests potential for contamination of groundwater (Section 2.3.3). Pb deposition to soils has decreased since the phase-out of leaded on-road gasoline (Section 2.3.3.1). Recent studies of metal concentrations in leaf litter and organic roadside debris suggest that the litter can act as a temporary sink for metals from the soil around and below leaves on the ground (Section 2.3.3.2). Leaching has been consistently observed to be a slower process for Pb than for other contaminants because Pb is only weakly soluble in pore water, but anthropogenic Pb is more available for leaching than naturally occurring Pb in soil (Section 2.3.3.3). Overall, recent research confirms the generally low mobility of Pb in soil. This limited mobility is strongly dependent on colloid amount and composition, as well as pH, and may be greater in some contaminated soils. Low mobility allows soils to act as a sink for atmospheric Pb potentially for decades or longer. Hence, atmospheric Pb concentrations that peaked several decades ago may still be present in soil in the absence of remediation.

Sources of Pb to surface waters include direct atmospheric deposition and indirect deposition via runoff and industrial discharge (Section 2.3.2). Because dispersal in waterways is a relatively rapid process, concentrations in surface waters are highest near sources of pollution before substantial Pb removal by flushing and sedimentation occurs. Transport in surface water is largely controlled by exchange with sediments, and the cycling of Pb between water and sediments is governed by chemical, biological, and mechanical processes that are affected by many factors, including salinity, organic

complexation, oxidation-reduction potential, and pH. Metals in waterways are transported primarily as soluble chelates and ions, or adsorbed on colloidal surfaces, including secondary clay minerals, Fe and Mn oxides or hydroxides, and organic matter, and adsorption on organic or inorganic colloids is particularly important for Pb. The extent of sorption strongly depends on particle size as smaller particles have larger collective surface areas. Pb is relatively stable in sediments, with long residence times and limited mobility (Section 2.3.2.1). As described in previous sections, Pb enters and is distributed in bodies of water largely in PM form. In rivers, particle-bound metals can often account for ≥ 75% of the total load (Section 2.3.2.2). The flux of Pb in aquatic ecosystems is therefore influenced by the dynamic physical and chemical interactions within a watershed.

Particles associated with runoff are mostly PM, with a relatively small dissolved fraction, and dissolution of carbonate and related compounds are important contributors to Pb pollution in runoff waters. Pb release into runoff is dependent on storm intensity and length of dry periods between rain events. A "first flush effect" occurs with highest runoff concentrations observed at the beginning of a rain event. Most recent studies have concluded that, during storm events, Pb is transported together with large PM. Some studies, however, found that Pb was concentrated in the fine PM fraction and, occasionally, Pb was found predominantly in the dissolved fraction. Since the 2006 Pb AQCD, snowmelt and rain-on-snow events are better understood, and it has been observed that greater runoff occurs from snowmelt and in rain on snow events than when snow is not present, and that metals, including Pb, are often associated with coarse PM under these circumstances. Runoff in rural areas is strongly controlled by soil type and the presence of vegetation, with less runoff and greater retention in mineral soils or when grass is present, and more runoff for soils high in organic matter (OM).

Sediments can be either a source or a sink for metals in the aquatic environment (Section 2.3.2). Release can be via re-suspension of the sediment bed via wind, wave, and tidal action or by dissolution from sediment to the water column. Sediment resuspension from marine environments is important, with disturbance of bed sediments by tidal action in estuarine areas resulting in a general greater capacity for re-suspension of PM. Recent research on Pb flux from sediments in natural waters has demonstrated that resuspended Pb is largely associated with OM or Fe and Mn particles, but that anoxic or depleted oxygen environments in sediments play an important role in Pb cycling. This newer research indicated that resuspension and release from sediments largely occurs during discrete events related to storms. It has also confirmed that resuspension is an important process that strongly influences the lifetime of Pb in bodies of water. Finally, there have been important advances in understanding and modeling of Pb partitioning in complex aquatic environments.

6.2.2 Ecosystem Exposure, Lag Time and Re-entrainment of Historically Deposited Pb

Ecosystem exposure from atmospheric emissions of Pb depends upon the amount of Pb deposited per unit time. Ecosystem response will also depend upon the form in which the Pb is deposited, the areal extent of such deposition, and modifying factors that affect Pb bioavailability in soil, sediments, and water (e.g., pH, organic matter) (Sections 6.3.2, 6.4.2 and 6.4.3). However, there is frequently a lag in time between when metals are emitted and when an effect is seen, particularly in terrestrial ecosystems and, to a lesser extent, in aquatic sediments. This is because the buffering capacity of soils and sediments permits Pb to become sequestered into organic matter, reducing its availability for uptake by organisms. The lag time from start of emissions to achieving a critical load can be calculated as the time to reach steady state after Pb was initially added to the system. Excluding erosion processes, the time required to achieve 95% of steady state is about 4 half-lives ($t_{1/2}$)[1] (Smolders et al., 2007). Conversely, once emissions cease, the same amount of time is required to reduce metal concentrations to background levels.

Time to steady state for metals in soils depends upon rates of erosion, uptake by plants, and leaching or drainage from soils. Ignoring erosion, half-life of metals can be predicted (Smolders et al., 2007) for a soil as:

$$t_{1/2} = \frac{0.69 \times d \times 10{,}000}{y \times TF + \frac{R}{\rho K_d}}$$

Equation 6-1

where:

d is the soil depth in meters (m)

y is the annual crop yield (tons/ha per year)

TF is the ratio of the metal concentration in plant to that in soil

R is the net drainage loss from the soil depth of concern (m^3/ha per year)

P is the bulk density of soil [kg(dry weight)/L]

K_d is the ratio of the metal concentration in soil to that in soil pore solution (L/kg)

[1] Time required to reduce the initial concentration by 50% if metal input is zero.

Metals removed by crops (or plants in general) comprise a very small fraction of the total soil metal and can be ignored for the purpose of estimating time to steady state. Thus, Equation 6-1 is simplified to:

$$t_{1/2} = \frac{0.69 \times d \times 10{,}000}{\frac{R}{\rho K_d}}$$

Equation 6-2

and becomes a function of soil depth, the amount of rainfall, soil density, and soil properties that affect K_d. Pb has a relatively long time to steady state compared to other metals, as shown in Table 6-1.

Table 6-1 Comparison among several metals: Time to achieve 95% of steady state metal concentration in soil; example in a temperate system.

Metal	Loading rate (g/ha·yr)	K_d (L/kg)	Time (years)
Se	100	0.3	1.3
Cu	100	480[a]	1,860[a]
Cd	100	690[a]	2,670[a]
Pb	100	19,000[a]	73,300[a]
Cr	100	16,700[a]	64,400[a]

[a]Mean K_d (ratio of total metal concentrations in soils to that in soil pore water); and Time to achieve 95% of steady-state concentration in soil. (49 Dutch soils) (de Groot et al., 1998).

Note: Based on a soil depth of 25 cm, a rain infiltration rate of 3,000 m^3/ha per yr, and the assumption that background was zero at the start of loading.

Source: Reprinted with permission of CRC Press, Smolders et al. (2007)

In aquatic systems, $t_{1/2}$ for Pb in the water column depends on the ratio of the magnitudes of the fluxes coming from and going into the sediment, the ratio of the depths of the water column and sediment, and the sediment $t_{1/2}$. Sediment $t_{1/2}$ is dependent upon the particulate and dissolved fractions and is calculated as for soils (Equation 6-2).

Re-entrainment of Pb particles via windblown dust from surface soils or dry sediments may occur. Amount and distance of re-entrained particles and deposition rates are dependent upon wind velocity and frequency; size, density, shape, and roughness of the particle; soil or sediment moisture; and terrain features including openness (including amount of vegetation), aspect relative to wind direction, and surface roughness. Resuspension is defined in terms of a resuspension factor, K, with units of ($meters^{-1}$), or a

resuspension rate (Λ), with units of seconds^{-1} (Equation 6-3). The resuspension rate, Λ, is the fraction of a surface contaminant that is released per time and is defined by:

$$\Lambda = \frac{R}{C}$$

Equation 6-3

where:

R is the upward resuspension flux (µg/m^2 per second),

C is the soil (or dry sediment) Pb concentration (µg/m^2).

Such emissions may have local impacts, but are not likely to have long-range effects, as particles generally remain low to the ground and are not lifted into the atmosphere. Although re-entrainment may alter the particle size distribution in a local area, it generally does not alter the bioavailable fraction, and deposited particles will be subject to the same biogeochemical forces affecting bioavailability. Therefore, exposure via re-entrainment should be considered additive to exposure from atmospheric particulate deposition in terrestrial and aquatic ecosystems.

6.2.3 Concentrations in Non-Air Media

Pb from multiple sources moves through environmental media as described in Section 6.2.1 and Figure 6-1 and has led to measurable Pb concentrations in soil, water, sediment and biota in terrestrial and aquatic ecosystems (Table 6-2). The highest concentrations of Pb in the environment are currently found near Pb sources, such as metal smelters and industrial processing. After phase-out of Pb from on-road gasoline, Pb concentrations have decreased considerably in rain, snowpack and surface waters. Declining Pb concentrations in tree foliage, trunk sections, and grasses, as well as surface sediments and soils in some locations, have also been observed (U.S. EPA, 2006b). In contrast, Pb is retained in soils and sediments, where it may provide a historical record of deposition and associated concentrations. In remote lakes, sediment profiles indicate higher Pb concentrations in near surface sediment as compared to pre-industrial era sediment from greater depth, with peak concentrations between 1960 and 1980 (when leaded on-road gasoline was at peak use).

Atmospheric deposition has led to measurable Pb concentrations observed in rain, snowpack, soil, surface waters, sediments, agricultural plants, livestock, and wildlife. Concentrations of Pb in moss, lichens, peat, and aquatic bivalves have been used to

understand spatial and temporal distribution patterns of air Pb concentrations. The amount of Pb in ecosystems is influenced by numerous factors, however, and it is not currently possible to determine the contribution of atmospherically-derived Pb from total Pb. Food, drinking water, and inhalation are likely major routes of exposure for livestock and terrestrial wildlife. Ingestion and water intake are the major routes of Pb exposure for aquatic organisms. In these exposure pathways, the bioavailable Pb may be from multiple sources. Information on ambient Pb concentrations in non-air media and biota is reported in Section 2.6, and concentrations considered in the interpretation of the ecological evidence are tabulated in Table 6-2.

The most extensive survey of background soil Pb concentration in the contiguous U.S. was conducted between 1961 and 1976, and comprised 1,319 non-urban, undisturbed sampling locations, where 250 cm^3 of soil was collected at a depth of 20 cm (Shacklette and Boerngen, 1984). The lower detection limit was 10 mg Pb/kg, and 14% of the 1,319 samples were below it. The mean Pb concentration was 19.3 mg Pb/kg, the median 15 mg Pb/kg, and the 95th percentile 50 mg/kg. Sixteen locations had Pb concentrations between 100 and 700 mg Pb/kg. These results were in agreement with 3 previous surveys. When creating the Ecological Soil Screening Level (Eco-SSL) guidance document, the U.S. EPA (2007d, 2003b) augmented these data with observations from an additional 13 studies conducted between 1982 and 1997, most of them limited to one state. The resulting data were summarized using state means for each of the fifty states. Those state means ranged between 5 and 38.6 mg Pb/kg, with an overall national mean of 18.9 mg Pb/kg. No new data on background concentrations of Pb in U.S. soils have been published since 2005.

Table 6-2 Pb concentrations in non-air media and biota considered for ecological assessment.

Media	Pb Concentration	Years Data Obtained	References
Soil (non-urban)	Contiguous U.S. Median: 15 mg Pb/kg (dry weight) Contiguous U.S. 95th Percentile: 50 mg Pb/kg (dry weight)	1961-1976	Shaklette (1984)
	National Average: 18.9 mg Pb/kg (dry weight) Range of state averages: 5-38.6 mg Pb/kg (dry weight)	1961-1997	U.S. EPA (2007d, 2006b, 2003b)
Freshwater Sediment	Median: 73 mg Pb/kg (dry weight)	1996-2001	Mahler et al. (2006)
	Median: 28 mg Pb/kg[b] (dry weight)	1991-2003	U.S. EPA (2006b)
Saltwater Sediment	Range: 0.6 to 1,050 mg Pb/kg[a]	Dates not available	Sadiq (1992)
Fresh Surface Water (Dissolved Pb)[b]	Median: 0.50 µg Pb/L[b]; Max: 30 µg Pb/L, 95th percentile 1.1 µg Pb/L	1991-2003	U.S. EPA (2006b)
	Range: 0.0003-0.075 µg Pb/L (Set of National Parks in western U.S.)	2002-2007	Field and Sherrell (2003), U.S. National Park Service (2011)
Saltwater[c]	Range: 0.01–27 µg Pb/L	Dates not available	Sadiq (1992)
Vegetation	Lichens: 0.3-5 mg Pb/kg (dry weight) (Set of National Parks in western U.S.)	2002-2007	U.S. National Park Service (2011)
	Grasses: Geometric Mean: 0.31 kg Pb/kg (dry weight)	1980s-2000s	Vandenhove et al. (2009)
Vertebrates	Fish: Geometric Mean: 0.59 mg Pb/kg (dry weight) (whole fish) Geometric Mean: 0.15 mg Pb/kg (dry weight) (liver) Range: 0.08-22.6 mg Pb/kg (dry weight) (whole fish) Range: 0.01-12.7 mg Pb/kg (dry weight) (liver)	1991-2003	U.S. EPA (2006b)
	Fish (from a set of national parks in western U.S.): 0.0033 (fillet) to 0.97 (liver) mg Pb/kg (dry weight) Moose[d,e]: 0.008-0.029 mg Pb/kg (dry weight) (meat) Moose[d,e]: 0.012-0.023 mg Pb/kg (dry weight) (liver)	2002-2007	U.S. National Park Service (2011)

[a]No information available regarding wet or dry weight

[b]Based on synthesis of National Water-Quality Assessment (NAWQA) data reported in 2006 Pb AQCD (U.S. EPA, 2006b)

[c]Data from a combination of brackish and marine saltwater samples. In general, Pb in seawater is higher in coastal areas and estuaries since these locations are closer to sources of Pb contamination and loading from terrestrial systems.

[d] The reference cited and its source citations show that observations date from studies published in 1977-1990, indicating that the data were obtained no later than those years. Further, these measurements seem to be for non-U.S. locations, including the max, which is well above other reported values in these refs.

[e]Three moose in one Alaskan park

The 2006 Pb AQCD reported representative median and range of Pb concentrations in surface waters (median 0.50 µg Pb/L, range 0.04 to 30 µg Pb/L) and sediments (median 28 mg Pb/kg dry weight, range 0.5 to 12,000 mg Pb/kg dry weight) in the U.S. based on a synthesis of National Water Quality Assessment (NAWQA) data (U.S. EPA, 2006c). In an additional study using data collected from 1996-2001 the median Pb concentration in sediment was reported to be 73 mg Pb/kg dry weight (Mahler et al., 2006). A range of 0.01 to 27 µg Pb/L for saltwater was reported by Sadiq although the values are not specific to the U.S. and include open sea areas as well as estuarine and coastal waters (Sadiq, 1992). In general, Pb in seawater is higher in coastal areas and estuaries since these locations are closer to sources of Pb contamination and loading from terrestrial systems (Sadiq, 1992).

Measured concentrations of Pb in soils, sediment and water are not necessarily representative of the amount of Pb that is bioavailable to plants, invertebrates and vertebrates. Both bioaccessibility and bioavailability (Sections 6.3.3, 6.4.3, and 6.4.11) of Pb are dependent upon the physical, chemical, and biological conditions under which an organism is exposed at a particular geographic location. Experimental exposures may be difficult to compare with exposures under natural field conditions in terrestrial and aquatic systems where a variety of abiotic and biotic modifying factors affect Pb toxicity.

6.3 Terrestrial Ecosystem Effects

6.3.1 Introduction to Effects of Pb on Terrestrial Ecosystems

Numerous studies of the effects of Pb on components of terrestrial systems were reviewed in the 1977 Pb AQCD, the 1986 Pb AQCD and the 2006 Pb AQCD. The focus of the present document is on studies published since the last AQCD. Many of those studies were conducted near stationary sources of atmospheric Pb such as metal industries and mines, or using soil collected near those sources. Increasing proximity to the source was often used to generate a gradient of increasing exposure. As may be expected, concentrations found in close proximity to those sources are many times greater than those found at most locations around the country (data on concentrations of Pb in U.S. soils are reviewed in Section 6.2.3 and summarized in Table 6-2), and as indicated in the present document's Preamble, concentrations within one to two orders of magnitude of current conditions were considered. In addition, it is important to note that in all studies where a gradient of multiple concentrations was used, effects increased with increasing concentration. This is an important aspect in determining causality (see Preamble), and therefore justifies inclusion of some studies with very high exposures.

Inclusion of those studies also provides potential data for establishing dose-response relationships, and predicting effects at all concentrations, including those found away from stationary sources. Finally, some studies at very high concentrations were used to provide mechanistic information on Pb toxicity, allow for comparison of Pb uptake across taxa, or demonstrate the wide range of sensitivity among closely-related species.

Concentrations used in studies where Pb was added to soil experimentally are difficult to relate to concentrations found in natural environments that have been exposed to Pb pollution. As reviewed in the following sections, there is ample evidence that multiple factors, many of them known but not quantified, interact with Pb concentration to produce responses of widely varying magnitude for similar concentrations, or similar responses for varying concentrations of Pb. Thus, experimental concentrations that appear relatively low may be most comparable to relatively high concentrations in natural soils, and vice-versa. The various factors that interact with Pb concentration, and the evidence for those interactions, are discussed in the following sections. However, the same justifications for inclusion apply to added-Pb experiments as they do to studies where proximity to sources is used to vary exposure: gradients of Pb concentrations create gradients of response, and they often provide information on underlying mechanisms of toxicity even if the concentrations cannot be easily compared to natural ones.

The literature on terrestrial ecosystem effects of Pb published since the 2006 Pb AQCD, is considered with brief summaries from the 1977 Pb AQCD, the 1986 Pb AQCD and the 2006 Pb AQCD, where relevant. Section 6.3 is organized to consider uptake of Pb and effects at the species level, followed by community and ecosystem level effects. Recent evidence for Pb effects on reproduction, growth, and survival in terrestrial plants, invertebrates, and vertebrates is summarized in Table 6-4. Alterations to reproduction, growth, and survival of terrestrial organisms can lead to changes at the community and ecosystem levels of biological organization such as decreased abundance, reduced taxa richness, and shifts in species composition (Section 6.1). Soil biogeochemistry of Pb is reviewed in Section 6.3.2. Section 6.3.3 considers the bioavailability and uptake of Pb by plants, invertebrates, and vertebrates in terrestrial systems. Biological effects of Pb on terrestrial ecosystem components including plants and lichen, invertebrates, and vertebrates (Section 6.3.4) are followed by data on exposure and response of terrestrial species (Section 6.3.5). Effects of Pb at the ecosystem level of biological organization are discussed in Section 6.3.6. Section 6.3 concludes with a discussion of critical loads in terrestrial systems (Section 6.3.7), soil screening levels (Section 6.3.8), characterization of sensitivity and vulnerability of ecosystem components (Section 6.3.9), and effects on ecosystem services (Section 6.3.10). Concentration of Pb in soil is expressed in mg Pb/kg

soil, and concentration in solutions applied to soil or extracted from soil is expressed in mg Pb/L solution.

6.3.2 Soil Biogeochemistry and its Influence on Bioavailability

According to data presented in the 2006 Pb AQCD (U.S. EPA, 2006b), the fraction of soil metal that is directly available to plants is the fraction found in soil pore water, even though the concentration of metals in pore water is generally small relative to bulk soil concentration. At any given bulk soil concentration, the amount of Pb dissolved in soil solution is controlled by at least six variables: (1) solubility equilibria; (2) adsorption-desorption relationship of total Pb with inorganic compounds (e.g., oxides of Al, Fe, Si, Mn; clay minerals); (3) adsorption-desorption reactions of dissolved Pb phases on soil organic matter; (4) pH; (5) cation exchange capacity (CEC); and (6) aging. Adsorption-desorption of Pb to soil solid phases is largely controlled by total metal loading. Therefore, areas with high Pb deposition will exhibit a lower fraction of total Pb partitioned to inorganic and organic matter. Decreasing soil pH, CEC, and organic matter have been strongly correlated to increases in the concentration of dissolved Pb species. Aging of metals in soils results in decreased amounts of labile metal as the Pb becomes incorporated into the soil solid phase (McLaughlin et al., 2010). Data from recent studies have further defined the impact of pH, CEC, organic matter (OM), and aging on Pb mobilization and subsequent bioavailability in soils.

6.3.2.1 pH, CEC and Salinity

Models of metal bioavailability calibrated from 500+ soil toxicity tests on plants, invertebrates, and microbial communities indicated that soil pH and CEC are the most important factors governing both metal solubility and toxicity (Smolders et al., 2009). The variability of derived EC_{50} values was most closely associated with CEC. Smolders et al. (2007) determined that 12 to 18 months of artificial aging of soils amended with metal decreased the soluble metal fraction by approximately one order of magnitude. Relatedly, lower soil pH in forest environments relative to adjacent agricultural land resulted in higher solubility, and the mobility of smelter-produced metals was found to be greater in forest than in agricultural lands (Douay et al., 2009). Further, decreasing the soil pH via simulated acid rain events increased naturally occurring Pb bioavailability in field tests (Hu et al., 2009b). Miretzky et al. (2007) also showed that the concentration of mobile Pb was increased in acidic soils, and discovered that Pb adsorption to sandy loam clay was a function of weak electrostatic bonds with charged soil surfaces and was influenced by Fe and Mn oxide. Dayton et al. (2006) and Bradham et al. (2006) used path

analysis to help identify the main determinants of both organism Pb content and responses from among multiple soil characteristics. In parallel studies with lettuce and earthworms, they amended an array of 21 soils with varying characteristics with the same amount of Pb (2,000 mg /kg as Pb nitrate), and found that in lettuce, the main determinant of both accumulation and biological responses was OC, with contribution from pH and Fe/Al oxides. These latter characteristics only influenced accumulation and responses through their own impact on CEC. In earthworms, the main determinant of accumulation was pH, with contribution from CEC, but only through its association with other variables including OC, Fe/Al oxides, and pH. The main determinant of reproductive effects in earthworms was Fe/Al oxides, while pH drove differential mortality between the various soils.

Salinity can also alter Pb mobility and bioavailability in soils. Application of $CaCl_2$, MgCl, or NaCl salts to field-collected soils containing 31 to 2,764 mg Pb/kg increased the proportion of mobile metal. As the strength of the salt application was increased from 0.006 to 0.3 M, the proportion of released Pb increased from less than 0.5% to over 2% for $CaCl_2$ and from less than 0.5% to over 1% for MgCl (Acosta et al., 2011). However, the majority of salinity-induced effects occurred in soils containing less than 500 mg Pb/kg, and the proportion of released Pb decreased with increasing total soil Pb concentrations. In addition, the authors noted that Pb release from soils under increasing salinity was reduced at higher carbonate concentrations, indicating that the effect of soil salinity on Pb release is dependent on still other soil factors. A sequential extraction procedure was employed by Ettler et al. (2005) to determine the mobility of different Pb fractions present in soils collected from a mining and smelting area in the Czech Republic. Five Pb fraction categories were identified: (Fraction A) exchangeable, (Fraction B) acid extractable (bound to carbonates), (Fraction C) reducible (bound to Fe and Mn oxides), (Fraction D) oxidizable (complexed with organic carbon), and (Fraction E) residual (silicates). Tilled agricultural soils were found to have decreased Pb, likely as a result of repeated cultivation, with the majority of Pb represented as the reducible Fraction C. Pb concentration in undisturbed forest soils, however, was largely present as the exchangeable fraction (A), weakly bound to soil OM. However, the validity of associating sequentially extracted fractions with discrete geochemical components has not been definitively established, and as a consequence, the association between fractionation and bioavailability remains uncertain.

6.3.2.2 Organic Matter

Organic matter decreases bioavailability of Pb, but as it is turned over and broken down, pedogenic minerals become more important in Pb sequestration (Schroth et al., 2008).

Shaheen and Tsadilas (2009) noted that soils with higher clay content, organic matter, total calcium carbonate equivalent, and total free sesquioxides also exhibited higher total Pb concentration, indicating that less Pb had been taken up by resident plant species. Huang et al. (2008) examined the re-mobilization potential of Pb in forest soils, and determined that mobilization of total Pb was strongly associated with dissolved organic matter (DOM). Groenenberg et al. (2010) used a non-ideal competitive adsorption Donnan model to explain the variability of organic matter binding affinity and uncertainties associated with metal speciation. They found that natural variations in fulvic acid binding properties were the most important variable in predicting Pb speciation. Guo et al. (2006b) determined that the -COOH and -OH groups associated with soil OM were important factors in Pb sequestration in soil, and Pb sorption was increased as pH was raised from 2 to 8. Because organic content increased the Pb sequestration efficiency of soils, OM content had an inhibitory effect on Pb uptake by woodlouse species *Oniscus asellus* and *Porcellio scaber* (Gál et al., 2008). Vermeulen et al. (2009) demonstrated that invertebrate bioaccumulation of Pb from contaminated soils was dependent on pH and OM, but that other unidentified habitat-dependent factors also contributed. The relationship of bioaccumulation and soil concentration was modified by pH and OM, and also by habitat type. Kobler et al. (2010) showed that the migration of atmospherically deposited Pb in soil matrices was strongly influenced by soil type, indicating that certain soil types may retain Pb for longer periods of time than others. In soils characterized by well-drained substrate and limestone bedrock, Pb concentration decreased over time, likely as a result of water drainage and percolation. The authors contrasted this observation with reports of prolonged residence time in humic soils, particularly at the lower depths of the humus layer. They theorized that the most significant Pb migration route was transportation of particulate-bound Pb along with precipitation-related flow through large soil pores.

A number of recent laboratory studies have further defined the relationship of soil biogeochemical characteristics and Pb uptake by plants. As noted above, Dayton et al. (2006) found through path analysis that the main determinant of both accumulation and biological responses in lettuce grown on amended soil was OC. As part of a metal partitioning study, Kalis et al. (2007) determined that not only did metal concentration in the soil solution decrease as pH increased, but pH-mediated metal adsorption at the root surface of *Lolium perenne* determined root Pb concentration, with concentration in the shoot correlated with root concentration. Interestingly, Kalis et al. (2007) and Lock et al. (2006) also observed that the influx of Pb in the water-soluble fraction had an impact on soil pH. In addition, 1 µM humic acid decreased root Pb concentration in *L. perenne* plants grown in 0.1 and 1 µM Pb solution, likely as a result of Pb complexation and sequestration with the added OM (Kalis et al., 2006). Ma et al. (2010) also reported that long-term agricultural cultivation can decrease the rate of Pb desorption in soil through a

gradual OM-enrichment. Phosphorous soil amendments equivalent to 35 mg P/kg soil were observed to reduce the quantity of DPTA-extractable Pb from an average of 19 and 24 mg Pb/kg in unamended soils to 12 to 15 mg Pb/kg in P-amended soils. As a result, maize and soybean seedlings accumulated significantly less Pb: average concentrations in soybean shoot and root ranged from 4.4 to 5.2 mg Pb/kg with P addition (versus 9.21 mg Pb/kg without), while maize shoot concentrations average between 4.8 to 5.3 mg Pb/kg in P-amended soils (as compared with 10.16 mg Pb/kg in controls) (Xie et al., 2011).

6.3.2.3 Aging

Smolders et al. (2007) defined aging as the process responsible for decreasing the bioavailability of metals in soils independently of their persistence. Smolders et al. (2009) reviewed the effects of aging of Pb in soils on the toxicity of Pb to plants and soil invertebrates, with aging achieved in most studies primarily by leaching amended soil, but also through natural binding and complexation. In nearly half of the Pb soil studies reviewed, responses that were observed with freshly amended soil could no longer be detected following soil leaching, indicating that aged soils likely contain less bioavailable Pb. The authors concluded that competitive binding between soil ligands and biotic ligands on plant roots or invertebrate guts can be used to model the relationship of observed availability and toxicity of metals in soils. Because this concept is the basis of the Biotic Ligand Model (BLM) (Section 6.3.3), the authors proposed a terrestrial BLM approach to estimate the risk of metals to terrestrial organisms. However, Antunes et al. (2006) noted that there were several key challenges involved in development of a terrestrial BLM applicable to plants, particularly the reliable measurement of free ion activities and ligand concentration in the rhizosphere, the identification of the organisms' ligands associated with toxicity, and the possible need to incorporate kinetic dissolution of metal-ligand complexes as sources of free ion. Further, Pb in aged field soils has been observed to be less available for uptake into terrestrial organisms, likely as a result of increased sequestration within the soil particles (Antunes et al., 2006). Magrisso et al. (2009) used a bioluminescent strain of the bacterium *Cupriavidus metallidurans* to detect and quantify Pb bioavailability in soils collected adjacent to industrial and highway areas in Jerusalem, Israel, and in artificial preparations corresponding to separate soil components (carbonates, Fe-oxides, clays, organic matter, and quartz) freshly spiked with varying concentrations of Pb. The bacterium was genetically engineered to give off the bioluminescent reaction as a dose-dependent response, and was inoculated in soil slurries for three hours prior to response evaluation. Spiked soil components induced the bioluminescent response, and field-collected components did not. However, the comparability of the simulated soils and their Pb concentration with the field-collected

samples was not entirely clear. Lock et al. (2006) compared the Pb toxicity to springtails (*Folsomia candida*) from both laboratory-spiked soils and field-collected Pb-contaminated soils of similar Pb concentrations. Total Pb concentrations of 3,877 mg Pb/kg dry weight and higher always caused significant effects on *F. candida* reproduction in the spiked soils. In field soils, only the soil with the highest Pb concentration of 14,436 mg Pb/kg dry weight significantly affected reproduction. When expressed as soil pore-water concentrations, reproduction was never significantly affected at Pb concentrations of 0.5 mg Pb/L, whereas reproduction was always significantly affected at Pb concentrations of 0.7 mg Pb/L and higher, independent of the soil treatment. Leaching soils prior to use in bioassays had only a slight effect on Pb toxicity to resident springtails, suggesting that among the processes that constitute aging of Pb in field soils, leaching is not particularly important with respect to bioavailability.

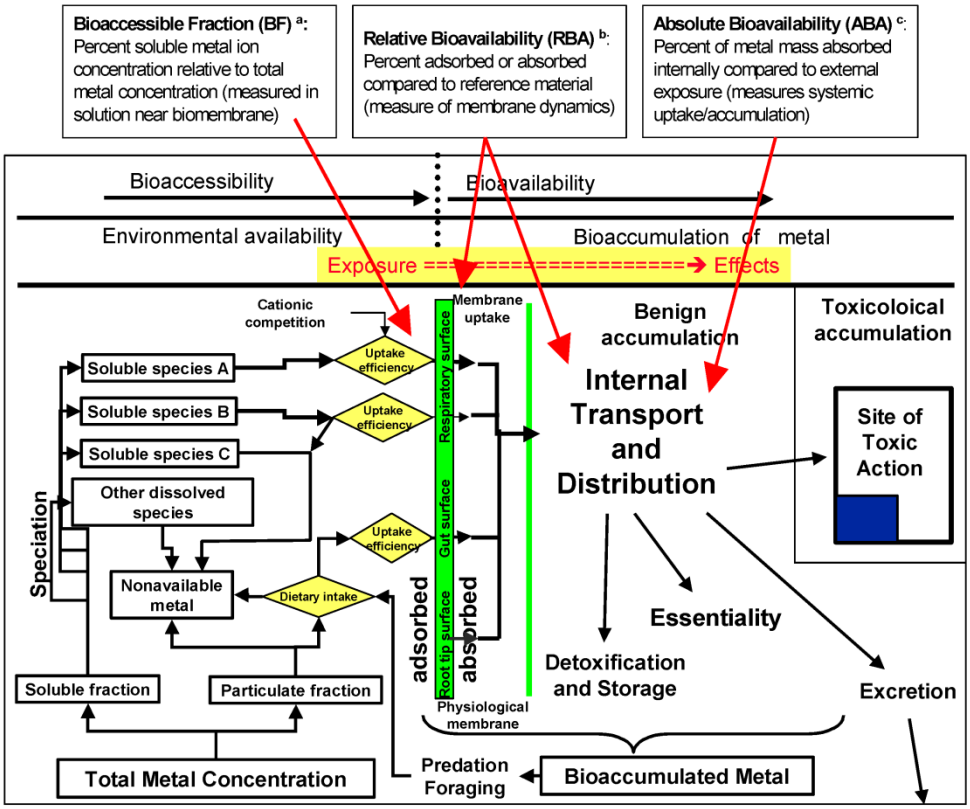
Red-backed salamanders (P*lethodon cinereus*) exposed to Pb-amended soils (553 mg Pb/kg, 1,700 mg Pb/kg, 4,700 mg Pb/kg, and 9,167 mg Pb/kg) exhibited lowered appetite and decreased white blood cell counts at the two highest concentrations, as compared to controls (Bazar et al., 2010). However, salamanders tolerated field-collected, aged soils containing Pb concentration of up to 16,967 mg Pb/kg with no significant deleterious effects.

In summary, studies published during the past 5 years continue to substantiate the important role that soil geochemistry plays in sequestration or release of Pb. Soil pH and CEC have long been known to be the primary controlling factors of the amount of bioavailable Pb in soils, and a recent review of more than 500 studies corroborates these findings (Smolders et al., 2009). Fe and Mn oxides are now known to also play an important role in Pb sequestration in soils. Pb binds to OM, although relatively weakly, and as the OM is broken down the Pb may be released into soil solution. Leaching of metal through soil pores may be the primary route for loss of bioavailable soil Pb; OM may reduce leaching and thus appear to be associated with Pb sequestration. Aging of Pb in soils (through incorporation of the metal into the particulate solid-phase of the soil) results in long term binding of the metal and reduced bioavailability of Pb to plants and soil organisms.

6.3.3 Bioavailability in Terrestrial Systems

Bioavailability was defined in the 2006 Pb AQCD as “the proportion of a toxin that passes a physiological membrane (the plasma membrane in plants or the gut wall in animals) and reaches a target receptor (cytosol or blood)” (U.S. EPA, 2006c). In 2007, EPA took cases of bioactive adsorption into consideration and revised the definition of

bioavailability as “the extent to which bioaccessible metals absorb onto, or into, and across biological membranes of organisms, expressed as a fraction of the total amount of metal the organism is proximately exposed to (at the sorption surface) during a given time and under defined conditions” (U.S. EPA, 2007c). The bioavailability of metals varies widely depending on the physical, chemical, and biological conditions under which an organism is exposed (U.S. EPA, 2007c). Characteristics of the toxicant itself that affect bioavailability are: (1) chemical form or species, (2) particle size, (3) lability, and (4) source. The bioavailability of a metal is also dependent upon the fraction of metal that is bioaccessible. As stated in the Framework for Metals Risk Assessment (U.S. EPA, 2007c), the bioaccessible fraction of a metal is the portion (fraction or percentage) of environmentally available metal that actually interacts at the organism’s contact surface and is potentially available for absorption or adsorption by the organism. The Framework states that “the bioaccessibility, bioavailability, and bioaccumulation properties of inorganic metals in soil, sediments, and aquatic systems are interrelated and abiotic (e.g., organic carbon) and biotic (e.g., uptake and metabolism) modifying factors determine the amount of an inorganic metal that interacts at biological surfaces (e.g., at the gill, gut, or root tip epithelium) and that binds to and is absorbed across these membranes. A major challenge is to consistently and accurately measure quantitative differences in bioavailability between multiple forms of organic metals in the environment.” A conceptual diagram presented in the Framework for Metals Risk Assessment (U.S. EPA, 2007c) summarizes metals bioavailability and bioaccumulation in aquatic, sediment and soil media (Figure 6-2).



[a]BF is most often measured using in vitro methods (e.g., artificial stomach), but it should be validated by in vivo methods.

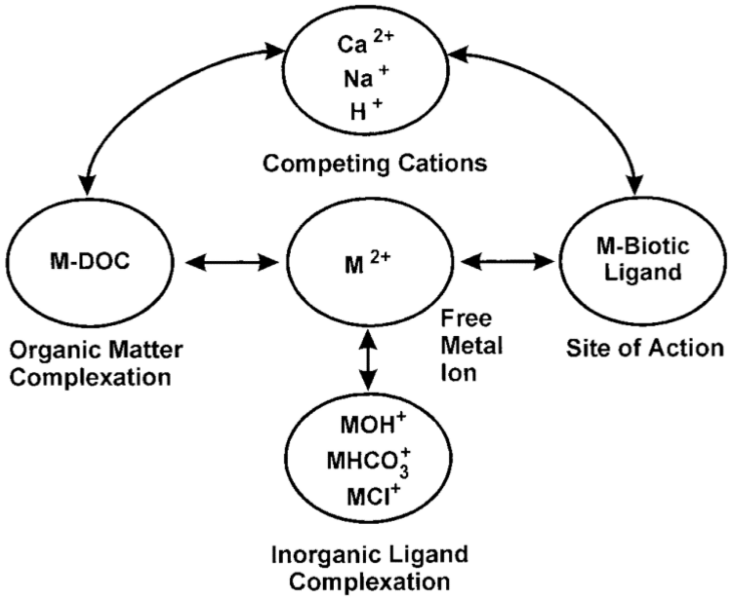
[b]RBA is most often estimated as the relative absorption factor, compared to a reference metal salt (usually calculated on the basis of dose and often used for human risk, but it can be based on concentrations).

[c]ABA is more difficult to measure and used less in human risk; it is often used in ecological risk when estimating bioaccumulation or trophic transfer.

Source: ERG (2004) and U.S. EPA (2007c).

Figure 6-2 Conceptual diagram for evaluating bioavailability processes and bioaccessibility for metals in soil, sediment, or aquatic systems.

The BLM attempts to integrate the principal physical and chemical variables that influence Pb bioavailability. The model considers the reactions of Pb with biological surfaces and membranes (the site of action) to predict the bioavailability and uptake of the metal (Figure 6-3), and integrates the binding affinities of various natural ligands and the biological uptake rates of organisms to predict both the bioaccessible and bioavailable fraction of Pb in the environment, and to determine the site-specific toxicity of the bioavailable fraction. In principle, the BLM can be used for determining toxicity in water, sediment, and soil media, however, the parameter values that influence BLM are, in general, characterized to a greater extent in aquatic systems than in terrestrial systems (Section 6.4.4).



Source: Reprinted with permission of John Wiley and Sons; from Di Toro et al. (2001)

Figure 6-3 Schematic diagram of the biotic ligand model.

New information on sources of Pb in terrestrial ecosystems, and their influence on subsequent bioavailability, was reviewed in Chapter 2, while new information on the influence of soil biogeochemistry on speciation and chemical lability was presented in Section 6.3.2. This section summarizes recent literature on uptake and subsequent presence of Pb in tissues. The 2006 Pb AQCD (U.S. EPA, 2006b) extensively reviewed the methods available for quantitative determination of the mobility, distribution, uptake, and fluxes of atmospherically delivered Pb in ecosystems, and they are not reviewed in this section. The 2006 Pb AQCD also reported bioaccumulation factors (BAF) and bioconcentration factors (BCF). BAF is defined as the field measurement of metal concentration in tissues, including dietary exposures, divided by metal concentration in environmental media (Smolders et al., 2007). BCF is defined as the same measurement carried out in artificial media in the laboratory that does not include dietary exposure (Smolders et al., 2007). The EPA Framework for Metals Risk Assessment states that the latest scientific data on bioaccumulation do not currently support the use of BCFs and BAFs when applied as generic threshold criteria for the hazard potential of metals (U.S. EPA, 2007c).

6.3.3.1 Terrestrial Plants

At the time of the 1977 Pb AQCD, it was understood that Pb uptake in plants was influenced by plant species and by the available Pb pool in the soils (U.S. EPA, 1977). The role of humic substances in binding Pb was better characterized by the 1986 Pb AQCD where it was stated that most plants cannot survive in soil containing 10,000 µg Pb/g (mg Pb/kg) dry weight if the pH is below 4.5 and the organic matter content is below 5% (U.S. EPA, 1986b). At the time of the 1986 AQCD, it was thought that Pb can be absorbed across the leaf surface into internal plant tissues, but that the vast majority of uptake is via roots (U.S. EPA, 1986b). The 2006 Pb AQCD (U.S. EPA, 2006b) noted that terrestrial plants accumulate atmospheric Pb primarily via two routes: direct stomatal uptake into foliage, and incorporation of atmospherically deposited Pb from soil into root tissue, followed by variable translocation to other tissues. Foliar Pb may include both incorporated Pb (i.e., from atmospheric gases or particles) and surficial particulate Pb deposition. Although the plant may eventually absorb the surficial component, its main importance is its likely contribution to the exposure of plant consumers. This section will first review recent studies on uptake of Pb by plants through foliar and soil routes, and their relative contribution, followed by the consideration of translocation of Pb from roots to shoots, including a discussion of variability in translocation among species. Data on ambient Pb levels associated with vegetation are summarized in Section 2.6.6.

Leaf and Root Uptake

Although Pb is not an essential metal, it is taken up from soils through the symplastic route, the same active ion transport mechanism used by plants to take up water and nutrients and move them across root cell membranes (U.S. EPA, 2006c). As with all nutrients, only the proportion of a metal present in soil pore water is directly available for uptake by plants. In addition, soil-to-plant transfer factors in soils enriched with Pb have been found to better correlate with bioavailable Pb soil concentration, defined as DTPA-extractable Pb, than with total Pb concentration (U.S. EPA, 2006c). Since the publication of the 2006 Pb AQCD, suggestive evidence has become available that a substantial proportion of Pb accumulated in shoots of some species of trees originates in direct leaf uptake of atmospheric Pb. Evidence for such direct uptake is weaker in herbaceous plants, and all data came from locations near stationary sources.

Field studies carried out in the vicinity of Pb smelters have determined the relative importance of direct foliar uptake and root uptake of atmospheric Pb deposited in soils. Hu and Ding (2009) analyzed ratios of Pb isotopes in the shoots of commonly grown vegetables and in soil at three distances from a smelter (0.1, 0.2, 5.0 km). Pb isotope

ratios in plants and soil were different at two of those locations, leading the authors to the conclusion that airborne Pb was being assimilated via direct leaf uptake. Soil Pb concentration in the rhizosphere at the three sites ranged between 287 and 379 mg Pb/kg (Site I), 155 and 159 mg Pb/kg (Site II), and 58 and 79 mg Pb/kg (Site III, selected as the control site). The median shoot and root Pb concentrations at each site were 36 and 47 mg Pb/kg, 176 and 97 mg Pb/kg, and 1.3 and 7 mg Pb/kg, respectively, resulting in shoot:root Pb ratios exceeding 1.0 in Site I (for Malabar spinach [*Basella alba*], ratio = 1.6, and amaranth [*Amaranthus spinosus*], ratio = 1.1), and in Site II (for the weeds *Taraxacum mongolicum*, ratio = 1.9, and *Rostellaria procumbens*, ratio = 1.7). However, the two species studied at Site II were not studied at Site I or Site III. In the control site (Site III), no plant was found with a Pb shoot:root ratio greater than 1.0. Hu and Ding (2009) concluded that metal accumulation was greater in shoot than in root tissue, which suggested both high atmospheric Pb concentration and direct stomatal uptake into the shoot tissue.

Cui et al. (2007) studied seven weed species growing in the vicinity of an old smelter (average soil Pb concentration of 4,020 mg Pb/kg) in Liaoning, China, to measure Pb accumulation rates in roots and shoots. Cutleaf groundcherry (*Physalis angulata*) accumulated the most Pb, with root and shoot concentration of 527 and 331 mg Pb/kg, respectively, and velvetleaf (*Abutilon theophrasti*) was the poorest absorber of Pb (root and shoot concentration of 39 and 61 mg Pb/kg, respectively). In all cases, weed species near the smelter accumulated more Pb than plants from non-polluted environments (5 mg Pb/kg), indicating that aerially deposited Pb produced by smelting is bioavailable to plants. However, the ratio of root:shoot Pb concentration varied by species, and the authors presented no data to differentiate Pb taken up from soil from Pb incorporated via foliar uptake. Angelova et al. (2010) examined Pb uptake by rapeseed plants (*Brassica napus*) grown in heavy metal contaminated soils 0.5 km and 15 km from the Non-Ferrous Metal Works, in Bulgaria. Average surface soil Pb concentration decreased with distance from the plant (200.3 and 24.6 mg Pb/kg, respectively), as did average DTPA-extractable Pb (69.7 and 4.9 mg Pb/kg, respectively). Pb content in stems and leaves in rapeseed grown at 0.5 km from the plant averaged 1.73 and 8.69 mg Pb/kg ; average stem and leaf Pb concentrations in rapeseed grown at the more distant location were reported as 0.72 and 1.42 mg Pb/kg, respectively (Angelova et al., 2010).

Pb plant BAFs for plants grown in 70 actively cropped fields in California averaged 0.052 for vegetable crops and 0.084 for grains; the highest reported Pb BAF (0.577) was found in onions. Authors compared the BAFs based on total Pb and Pb in solution and determined that both were accurate predictors of plant uptake (Chen et al., 2009b). Likewise, Zhang et al. (2011b) compiled Pb uptake data for several crop species in China, and reported an average BAF for grains (rice) of 0.009 (0.0009-0.03) and

0.41(0.0007-0.17) for leafy vegetables, such as spinach, Chinese cabbage and celery (Zhang et al., 2011b). Chrastny et al. (2010) characterized the Pb contamination of an agricultural soil in the vicinity of a shooting range. Pb was predominantly in the form of PbO and $PbCO_3$, and Pb was taken up by plants through both atmospheric deposition onto the plant and by root uptake.

The Pb content of ripe date palm (*Phoenix dactylifera*) fruit collected in Riyadh, Saudi Arabia was determined to be indicative of areas of heavy industrialization and urbanization; Pb concentrations in fruit flesh ranged from 0.34 to 8.87 mg Pb/kg dry weight, with the highest Pb date concentrations detected near freeways and industrial areas (Aldjain et al., 2011). Likewise, Pb concentrations in rosemary (*Rosmarinus officinalis*) flowers, stems, and leaves were significantly higher in the urban areas of Al-Mafraq and Irbid, Jordan than in the smaller town of Ma'an, Jordan (53.6 to 86.5 mg Pb/kg versus 16.2 to 16.7 mg Pb/kg). Authors noted a significant difference between Pb concentrations in washed and unwashed rosemary samples, indicating that aerial deposition and surface dust is likely a significant source of plant-associated Pb (El-Rjoob et al., 2008).

Bilberry (*Vaccinium myrtillus*), accumulated the highest amount of Pb out of four total herbaceous species growing in Slovakian spruce ecosystems with variable soil Pb concentrations, giving BAFs of 0.09 to 0.44, depending on location (Kuklova et al., 2010). Because of their long life spans, trees can provide essential information regarding the sources of bioavailable Pb. A Scots pine forest in northern Sweden was found to incorporate atmospherically derived Pb pollution directly from ambient air, accumulating this Pb in bark, needles, and shoots (Klaminder et al., 2005). Nearly 50% of total tree uptake was estimated to be from direct adsorption from the atmosphere, as determined using isotopic ratios and a binary mixing model. Further, Aznar et al. (2009a) found that the Pb content of black spruce (*Picea mariana*) needles collected along a metal contamination gradient emanating from a Canadian smelter in Murdochville, Quebec, showed a significant decrease in Pb concentration with increasing distance from the smelter. Interestingly, older needles were determined to accumulate larger quantities of Pb than younger ones. Foliar damage and growth reduction were also observed in the trees (Aznar et al., 2009a). They were significantly correlated with Pb concentration in the litter layer. In addition, there was no correlation between diminished tree growth and Pb concentration in the deeper mineral soil layers, strongly suggesting that only current atmospheric Pb was affecting trees (Aznar et al., 2009b). Similarly, Kuang et al. (2007) noted that the Pb concentration in the inner bark of *Pinus massoniana* trees growing adjacent to a Pb-Zn smelter in the Guangdong province of China was much higher (1.87 mg Pb/kg dry weight) than in reference-area trees. Because concentration in the

inner bark was strongly correlated with concentration in the outer bark, they concluded that the origin of the Pb was atmospheric.

Dendrochronology (tree ring analysis) has become an increasingly important tool for measuring the response of trees to Pb exposure (Watmough, 1999). Tree ring studies reviewed in the 1977 Pb AQCD showed that trees could be used as indicators of increasing environmental Pb concentrations with time. Additional studies in the 1986 Pb AQCD indicated that Pb could be translocated from roots to the upper portions of the plant and that the amounts translocated are in proportion to concentrations of Pb in soil (U.S. EPA, 1986b). The advent of laser ablation inductively coupled plasma mass spectrometry has made measurement of Pb concentration in individual tree rings possible (Witte et al., 2004; Watmough, 1999). This allows for close analysis of the timing of Pb uptake relative to smelter activity and/or changes in soil chemistry. For example, Aznar et al. (2008a) measured Pb concentration in black spruce tree rings to determine the extent and timing of atmospheric deposition near the Murdochville smelter. Variability in tree-ring Pb content seemed to indicate that trees accumulated and sequestered atmospheric Pb in close correlation with the rates of smelter emission, but that sequestration lagged about 15 years behind exposure. However, the ability to determine time of uptake from the location in growth rings is weakened in species that transfer Pb readily from outer bark to inner bark. Cutter and Guyette (1993) identified species with minimal radial translocation from among a large number of tree species, and recommended the following temperate zone North American species as suitable for metal dendrochronology studies: white oak (*Quercus alba*), post oak (*Q. stellata*), eastern red cedar (*Juniperus virginiana*), old-growth Douglas fir (*Pseudotsuga menziesii*), and big sagebrush (*Artemisia tridentata*). In addition, species such as bristlecone pine (Pinus aristata), old-growth redwood (*Sequoia sempervirens*), and giant sequoia (*S. gigantea*) were deemed suitable for local purposes. Patrick and Farmer (2006) determined that European sycamore (*Acer pseudoplatanus*) are not suitable for this type of dendrochronological analysis because of the formation of multiple annual rings.

Pb in sapwood and heartwood is more likely a result of soil uptake than of direct atmospheric exposure (Guyette et al., 1991). Differentiation of geogenic soil Pb in tree tissue from Pb that originated in the atmosphere requires measurement of stable Pb isotope ratios (Patrick, 2006). Tree bark samples collected from several areas of the Czech Republic were subjected to stable Pb isotope analysis to determine the source and uptake of atmospheric Pb (Conkova and Kubiznakova, 2008). Results indicated that beech bark is a more efficient accumulator of atmospheric Pb than spruce bark. A decrease in the $^{206}Pb/^{207}Pb$ ratio was measured in bark and attributed to increased usage of leaded gasoline between 1955 and 1990; an increased $^{206}Pb/^{207}Pb$ ratio was ascribed to coal combustion (Conkova and Kubiznakova, 2008). Similarly, Savard et al. (2006)

compared isotope ratios of $^{206}Pb/^{207}Pb$ and $^{208}Pb/^{206}Pb$ in tree rings from spruce trees sampled at a control site near Hudson Bay, with those sampled near the Horne smelter active since 1928, in Rouyn-Noranda, Canada. The concentration of total Pb showed a major increase in 1944 and a corresponding decrease of the $^{206}Pb/^{207}Pb$ ratios, suggesting that the smelter was responsible for the increased Pb uptake (Savard et al., 2006). The authors suggested that the apparent delay of 14 years may have been attributable to the residence time of metals in airborne particles the buffering effect of the soils and, to a lesser extent, mobility of heavy metals in tree stems. Furthermore, through the use of the two different isotope ratios, Savard et al. (2006) were able to differentiate three types of Pb in tree rings: natural (derived from the mineral soil horizons), industrial (from coal burning urban pollution), and mining (typical of the volcanogenic massive sulfide ore deposits treated at the Horne smelter).

Devall et al. (2006) measured Pb uptake by bald-cypress trees (*Taxodium distichum*) growing in a swamp near a petroleum refinery and along a bank containing Pb-contaminated dredge spoils. They measured Pb in tree cores and showed greater uptake of Pb by trees in the swamp than by trees growing on the dredge spoil bank, attributing the difference to exposure source (refinery versus dredge spoils) and differences in soil chemistry between the swamp and the dredge spoil bank (Devall et al., 2006). Similarly, Gebologlu et al. (2005) found no correlation between proximity to roadway and accumulated Pb in tomato and bean plants at sites adjacent to two state roads in Turkey (average Pb concentration 5.4 and 6.0 mg Pb/kg), indicating that uptake may be influenced by multiple factors, including wind direction, geography, and soil chemistry. Average Pb levels in leaves were 0.6 and 0.5 mg Pb/kg for tomato and bean plants, respectively, while fruit concentration averaged 0.4 mg Pb/kg for both species. Conversely, if foliar contamination is due primarily to dust deposition, distance from a source such as a road may be easily correlated with Pb concentration on the plants. For example, Ai-Khlaifat and Al-Khashman (2007) collected unwashed date palm (*Phoenix dactylifera*) leaves at 3-meter trunk height from trees in Jordan to assess the extent of Pb contamination from the city of Aqaba. Whereas relatively low levels of Pb were detected in leaves collected at background sites (41 mg Pb/kg), leaves collected adjacent to highway sites exhibited the highest levels of Pb (177 mg Pb/kg). The authors determined that Pb levels in date palm leaves correlated with industrial and human activities (e.g., traffic density) (Ai-Khlaifat and Al-Khashman, 2007). Likewise, Pb concentrations were significantly enriched in tree bark samples and road dust collected in highly urbanized areas of Buenos Aires, Argentina (approximate average enrichment factors of 30 and 15 versus reference samples) (Fujiwara et al., 2011). However, decreases in tissue Pb concentration with increasing distance from stationary sources can also follow from decreasing Pb in soil. Bindler et al. (2008) used Pb isotopes to assess the relative importance of pollutant Pb versus natural Pb for plant uptake and cycling in Swedish

forested soils. The Pb isotopic composition of needles/leaves and stemwood of different tree species and ground-cover plants indicated that the majority of Pb present in these plant components was derived from the atmosphere, either through aerial interception or actual uptake through the roots. For the ground-cover plants and the needles/leaves, the $^{206}Pb/^{207}Pb$ isotopic ratios (1.12 to 1.20) showed that the majority of Pb was of anthropogenic origin. Stemwood and roots have higher $^{206}Pb/^{207}Pb$ ratio values (1.12 to 1.30) which showed the incorporation of some natural Pb as well as anthropogenic Pb. For pine trees, the isotopic ratio decreased between the roots and the apical stemwood suggesting that much of the uptake of Pb by trees is via aerial exposure. Overall, it was estimated that 60-80% of the Pb in boreal forest vegetation originated from pollution; the Pb concentrations were, however, quite low – not higher than 1 mg Pb/kg plant material, and usually in the range of 0.01-0.1 mg Pb/kg plant material (while soils had a range of 5 to 10 mg Pb/kg in the mineral horizons and 50 to 150 mg Pb/kg in the O horizons). Overall, the forest vegetation recycles very little of the Pb present in soils (and thus does not play a direct role in the Pb biogeochemical cycle in boreal forest soils).

Fungal species, as represented by mushrooms, accumulate Pb from soils to varying degrees. Based on the uptake of naturally occurring ^{210}Pb, Guillen et al. (2009) established that soil-associated Pb was bioavailable for uptake by mushrooms, and that the highest ^{210}Pb accumulation was observed in *Fomes fomentarius* mushrooms, followed by *Lycoperdon perlatum*, *Boletus aereus*, and *Macrolepiota procera*, indicating some species differences. Benbrahim et al. (2006) also showed species differences in uptake of Pb by wild edible mushrooms, although they found no significant correlations between Pb content of mushrooms and soil Pb concentration. Pb concentrations in mushroom carpophores ranged from 0.4 to 2.7 mg Pb/kg from sites with soil concentrations ranging from 3.6 and 7.6 mg Pb/kg dry soil. Likewise, Semreen and Aboul-Enein (2011), reported the heavy metal uptake of wild edible mushrooms collected in various mountainous regions of Jordan. Pb BCFs ranged between 0.05 *(Russula delica*) and 0.33 (*Bovista plumbea)* for six mushroom species. Pb BAFs for edible mushrooms collected from quartzite acidic soils in central Spain (containing 19.2 mg Pb/kg) ranged from 0.07 (*Macrolepiota procera*) to 0.45 (*Lepista nuda*) (Campos and Tejera, 2011).

Translocation and Sequestration of Pb in Plants

In the 1977 Pb AQCD it was recognized that most Pb taken up from soil remains in the roots and that distribution to other portions of the plant is variable among species (U.S. EPA, 1977). The 2006 Pb AQCD (U.S. EPA, 2006b) stated that most of the Pb absorbed from soil remains bound in plant root tissues either because (1) Pb may be deposited within root cell wall material, or (2) Pb may be sequestered within root cell organelles. More recent research largely confirms that Pb taken up from soil largely remains in roots,

but suggests that some species translocate meaningful amounts into shoot tissue. Sequestration of Pb may be a protective mechanism for the plant. Recent findings have been consistent with this hypothesis: Han et al. (2008) observed Pb deposits in the cell walls and cytoplasm of malformed cells of *Iris lactea* exposed to 0 to 10 mM Pb (0 to 2,072 mg Pb/L) solution in sand culture for 28 days. They hypothesized that preferential sequestration of Pb in a few cells, which results in damage to those cells, helps in maintaining normal overall plant activities through the sacrifice of a small number of active cells. Similarly, macroscopic analysis of the roots of broad bean (*Vicia faba*) cultivated in mine tailings (average Pb concentration of 7,772 mg Pb/kg) by Probst et al. (2009) revealed dark ultrastructural abnormalities that were demonstrated to be metal-rich particles located in or on root cell walls. It is unclear whether the presence of these structures had any effect on overall plant health.

Clark et al. (2006) investigated Pb bioavailability in garden soils in Roxbury and Dorchester, MA. The sources of Pb were considered to be Pb from paints and from leaded gasoline additives, with 40 to 80% coming from paint. The average Pb concentration in foliar tissue of bean plants was 14 ± 5 mg Pb/kg while the concentration in the bean pod was only 20.6 mg Pb/kg. For mustard plants, there was a linear relationship (R^2=0.85) between Pb concentration in plant tissues and Pb concentration in the soil (both for plants grown in situ and those grown under greenhouse conditions).

Murray et al. (2009) investigated the uptake and accumulation of Pb in several vegetable species (carrot [*Daucus carota*], radish [*Raphanus sativus*], lettuce [*Lactuca sativa*], soybean [*Glycine max*], and wheat [*Triticum aestivum*]) from metal-contaminated soils, containing 10 to 40 mg Pb/kg and demonstrated that most Pb remained in the roots. No Pb was measured in the above-ground edible soybean and wheat tissues, while carrots, the most efficient accumulator of Pb, contained a maximum Pb tissue concentration of 12 mg Pb/kg dry mass. Similarly, (Cho et al., 2009) showed that green onion (*Allium fistulosum*) plants also take up little Pb when planted in soil spiked with Pb nitrate. No plant tissues contained a Pb concentration greater than 24 mg Pb/kg when grown for 14 weeks in soils of up to 3,560 mg Pb/kg, and the majority of bioavailable Pb was determined to be contained within the roots. Chinese spinach (*Amaranthus dubius*) also translocates very little Pb to stem and leaf tissue, and uptake from Pb-containing soils (28 to 52 mg Pb/kg) is minimal (Mellem et al., 2009). Wang et al. (2011c) determined tissue-specific BCFs for wheat grown in soils containing 93 to 1,548 mg Pb/kg. Although the average calculated root BCF was 0.3, very little Pb was translocated to shoots (average BCF=0.02), shells (0.006), and kernels (0.0007) (Wang et al., 2011c). However, it is not known how much of the Pb applied was present in pore water. Sonmez et al. (2008) reported that Pb accumulated by three weed species (*Avena sterilis*, *Isatis tinctoria*,

Xanthium strumarium) grown in Pb-spiked soils was largely concentrated in the root tissues, and little was translocated to the shoots (Sonmez et al., 2008).

The Pb BCFs for alfalfa (*Medicago sativa*) and crimson clover (*Trifolium incarnatum*) grown in mixtures of heavy metals (Pb concentrations of 10 to 500 µg Pb/kg) were reportedly low. For alfalfa, BCFs ranged from 0.02 to 0.12, while for crimson clover, these values were between 0.04 and 0.06 (Comino et al., 2011). The low shoot-root translocation factors reported for alfalfa (0.17 to 0.43) indicated that plant Pb content was largely contained in root tissue. Businelli et al. (2011) calculated whole-plant Pb BAFs for lettuce, radish, tomato and Italian ryegrass using Pb-spiked soils (average values of 0.025, 0.021, 0.032, and 0.65, respectively). Again, the majority of accumulated Pb was stored in root tissue, with comparatively little translocated to above-ground tissues (Businelli et al., 2011).

Recent research has shown that Pb translocation to stem and leaf tissues does occur at significant rates in some species, including the legume *Sesbania drummondii* (Peralta-Videa et al., 2009) and buckwheat (*Fagopyrum esculentum*) (Tamura et al., 2005). Wang et al. (2006b) noted that Pb soil-to-plant transfer factors were higher for leafy vegetables (Chinese cabbage, pak-choi, and water spinach) than for the non-leafy vegetables tested (towel gourd, eggplant, and cowpea). Tamura et al. (2005) demonstrated that buckwheat is an efficient translocator of Pb. Buckwheat grown in Pb-containing soils collected from a shooting range site (average 1M HCl extractable Pb= 6,643 mg Pb/kg) preferentially accumulated Pb in leaves (8,000 mg Pb/kg) and shoots (4,200 mg Pb/kg), over root tissues (3,300 mg Pb/kg). Although plant growth was unaffected, this level of leaf and shoot accumulation is likely to have significant implications for exposure of herbivores. Similarly, Shaheen and Tsadilas (2009) reported that vegetables (pepper, okra, and eggplant) grown in soils containing 24 to 30 mg Pb/kg total Pb were more likely to accumulate Pb in leaves (range: undetected to 25 mg Pb/kg) rather than in fruits (range: undetected to 19 mg Pb/kg); however, no significant correlation between soil Pb concentration and plant tissue Pb concentration could be established (Shaheen and Tsadilas, 2009). Tobacco plants were also observed to take up significant amounts of Pb into leaf tissue. Field-grown plants in soils containing an average of 19.8 mg Pb/kg contained average lower, middle and upper leaf Pb concentrations of 11.9, 13.3, and 11.6 mg Pb/kg respectively (Zaprjanova et al., 2010). Uptake by tobacco plants was correlated with both total soil Pb concentrations and the mobile Pb fraction (average 3.8 mg Pb/kg soil).

There is broad variability in uptake and translocation among plant species, and interspecies variability has been shown to interact with other factors such as soil type. By studying multiple species in four Pb-Zn mining sites in Yunnan, China, Li et al. (2009d)

demonstrated not only significant differences in uptake and translocation among the species studied, but also modification of the effect on species by type of soil. Plants sampled represented nine species from four families—Caryophyllaceae, Compositae, Cruciferae, and Pteridaceae. Overall, soil Pb concentration averaged 3,772 mg Pb/kg dry weight, with the highest site average measured at the Minbingying site (5,330 mg Pb/kg), followed by Paomaping (2,409 mg Pb/kg), Jinding (1,786 mg Pb/kg), and Qilinkeng (978 mg Pb/kg). The highest average shoot Pb concentration (3,142 mg Pb/kg) was detected in *Stellaria vestita* (Caryophyllaceae) collected at Paomaping, while *Sinopteris grevilloides* (Pteridaceae) collected from Minbingying exhibited the lowest shoot Pb concentration (69 mg Pb/kg). A similar trend was detected in root tissues. *S. vestita* root collected from the Paomaping area contained the maximum Pb concentration measured (7,457 mg Pb/kg), while the minimum root Pb levels were measured in *Picris hieracioides* (Pteridaceae) tissues collected from Jinding. These results indicate significant interspecies differences in Pb uptake, as well as potential soil-specific differences in Pb bioavailability. *S. vestita*, in particular, was observed to be an efficient accumulator of Pb, with a maximum enrichment coefficient of 1.3. Significant correlations between soil Pb concentration and average shoot and root Pb levels were also established (Li et al., 2009d). Within plant species, the variability in uptake and translocation of Pb may extend to the varietal level. Antonious and Kochhar (2009) determined uptake of soil-associated Pb for 23 unique genotypes from four species of pepper plants (*Capsicum chinense*, *C. frutescens*, *C. baccatum*, and *C. annum*). Soil Pb concentration averaged approximately 0.6 mg Pb/kg dry soil. No Pb was detected in the fruits of any of the 23 genotypes, except two out of seven genotypes of *C. baccatum*, which had 0.9 and 0.8 mg Pb/kg dry weight Pb in fruit.

Recent studies substantiated findings from the 2006 Pb AQCD that plants store a large portion of Pb in root tissue. Pb soil-to-plant transfer factors are higher for leafy vegetables than for the non-leafy vegetables (Wang et al., 2006b) and buckwheat has recently been shown to be an efficient translocator of Pb from soil to above-ground shoots (Tamura et al., 2005).

Field studies carried out in the vicinity of Pb smelters (Hu et al., 2009b) show that Pb may accumulate in shoot tissue through direct stomatal uptake rather than by soil-root-shoot translocation. For instance, Hovmand and Johnsen (2009) determined that about 98% of Pb sequestered in Norway spruce needles and twigs was derived from atmospheric sources, and that less than 2% of Pb was translocated from the roots (Hovmand et al., 2009). Dendrochronology has become more advanced in recent years and is a useful tool for monitoring historical uptake of Pb into trees exposed to atmospheric or soil Pb. Trees accumulate and sequester atmospheric Pb in close correlation with the rate of smelter emissions, although one study indicated that

sequestration can lag behind exposure from emissions by 15 years. Pb in the outer woody portion of the tree is more likely the result of direct atmospheric exposure, while Pb in sapwood is more likely a result of soil uptake. This difference provides an important tool for analyzing source apportionment of Pb accumulation in plants (Guyette et al., 1991).

6.3.3.2 Terrestrial Invertebrates

At the time of publication of the 2006 Pb AQCD (U.S. EPA, 2006b), little information was available regarding the uptake of atmospheric Pb pollution (direct or deposited) by terrestrial invertebrate species. Consequently, few conclusions could be drawn concerning the Pb uptake rate of particular species although there was some evidence that dietary or habitat preferences may influence exposure and uptake. Recent literature indicates that invertebrates can accumulate Pb from consuming a Pb-contaminated diet and from exposure via soil, and that uptake and bioaccumulation of Pb by invertebrates is lower than that observed for other metals.

Snails

Pauget et al. (2011) reported that uptake of Pb from soil by the land snail (*Cantareus asperses*) was most significantly influenced by soil pH and organic matter, as increases in these variables were correlated to decreased Pb bioavailability. *Cantareus asperses* snails exposed to dietary Pb at 3.3, 86, and 154 mg/kg of diet (spiked with Pb sulfate) for up to 64 days were found to assimilate a significant proportion of Pb, and feeding rates were unaffected by the presence of the metal (Beeby and Richmond, 2010). While BCFs for Cd were observed to increase over the 64-day study period, the rate of Pb assimilation remained consistent over time and the authors inferred the absence of a regulatory mechanism for uptake of Pb. The authors speculated that uptake is a function of growth or cell turnover instead. *Helix aspersa* snails rapidly accumulated Pb from contaminated soil (1,212 mg Pb/kg) and from eating contaminated lettuce (approximately 90 mg Pb/kg after 16 weeks' growth on Pb-contaminated soil) during the first 2 weeks of exposure, at which point snail body burdens reached a plateau (Scheifler et al., 2006b). There were no observed effects of Pb exposure or accumulation on survival or growth in *C. asperses* or *H. aspersa*. In another study (Ebenso and Ologhobo, 2009b), juvenile *Achatina achatina* snails confined in cages on former Pb-battery waste dump sites were found to accumulate Pb from both plant and soil sources. Soil Pb concentration averaged 20, 200, and 1,200 mg Pb/kg at the three main waste sites, while leaf tissues of radish (*Raphanus sativus*) grown at these sites averaged 7, 30, and 68 mg Pb/kg dry weight, respectively. Concentration of Pb in snail tissues rose with concentration in both soil and plants, and

the authors found that for both sources, a log-log relationship could be estimated with a very close fit (r^2 =0.94 and 0.95, respectively). Pb concentration in snail tissues averaged 12, 91, and 468 mg Pb/kg, respectively, at the three sites, which the authors stipulated were above the maximum permissible concentration of Pb for human consumption of mollusks, mussels, and clams (1.5 mg Pb/kg tissue) as determined by the U.K. Food Standards Agency. Pb concentration in snail tissues generally is much lower than that of the soil substrates upon which they were reared, but higher than in other soil-dwelling organisms. De Vaufleury et al. (2006) exposed *Helix aspersa* snails to standardized (International Organization for Standardization methodology [ISO 11267:1999]) artificial-substrate soils amended with sewer sludge containing 13, 26, 39, or 52 mg Pb/kg for 28 days without supplemental food. After the exposure period, snail foot tissue contained increased levels of Pb—1.9, 1.7, and 1.5 mg Pb/kg dry weight versus concentration averaging 0.4 mg Pb/kg in control organisms. Viscera also exhibited increased Pb levels at the two highest exposures, with measured tissue concentration of 1.2 and 1.1 mg Pb/kg, respectively, as compared with control tissue Pb levels of 0.4 mg Pb/kg. However, there was no significant increase in snail-tissue Pb concentration when natural soil was used in place of ISO medium, and there was no relationship between soil Pb concentration and snail tissue concentration, strongly suggesting the presence of soil variables that modify bioavailability. Notten et al. (2008) investigated the origin of Pb pollution in soil, plants, and snails by means of Pb isotope ratios. They found that a substantial proportion of Pb in both plants and snails was from current atmospheric exposure.

Finally, a study by Coeurdassier et al. (2007) found that the presence of snails was associated with higher Pb content in earthworms, suggesting that snails themselves may have an effect on bioavailability.

Earthworms

Accumulation studies conducted with *Eisenia* sp. earthworms documented the difficulty of extrapolating accumulation kinetic constants from one soil type to another, and showed that many soil physiochemical properties, including pH, organic matter, and CEC, among others, affect metal bioavailability (Nahmani et al., 2009). Source of Pb, and proportion of soil:leaf litter also affect Pb bioavailability. Bradham et al. (2006) examined the effect of soil chemical and physical properties on Pb bioavailability. *Eisenia andrei* earthworms were exposed to 21 soils with varying chemical and physical properties that were freshly spiked with Pb to give a standard concentration of 2,000 mg Pb/kg dry weight. At equivalent Pb exposure, the main determinants of both internal earthworm Pb concentration and mortality were pH first (with lower pH resulting in higher concentration and mortality), then CEC. However, the apparent importance of

CEC was due to its correlation with several other less important soil characteristics. These data corroborate that Pb bioavailability and toxicity are increased in acidic soils and in soils with a low CEC (Section 6.3.2). This finding was confirmed by Gandois et al. (2010), who determined that the free-metal-ion fraction of total Pb concentration in field-collected soils was largely predicted by pH and soil Fe content.

The role of soil profile and preferred depth was studied using eight species of earthworms from 27 locations in Switzerland, representing three ecophysiological groups (Ernst et al., 2008): epigeic (surface-dwelling worms), endogeic (laterally burrowing worms that inhabit the upper soil layers), and anecic (vertically burrowing worms that can reach depths of 2 meters). For epigeic and anecic earthworms, the total concentration of Pb in leaf litter and in soil, respectively, were the most important drivers of Pb body burdens. By contrast, the level of Pb in endogeic earthworms was largely determined by soil pH and CEC. As a result of these differences, the authors suggested that atmosphere-sourced Pb may be more bioavailable to epigeic than endogeic species, because it is less dependent on modifying factors. Suthar et al. (2008), on the other hand, found higher Pb bioaccumulation in the endogeic earthworm *Metaphire posthuma* than in the anecic earthworm species *Lampito mauritii*, and speculated that differences in Pb tissue level arose from differing life-history strategies, such as feeding behaviors, niche preferences, and burrowing patterns, all of which exposed the endogeic species to greater Pb concentration. Garg et al. (2009) reported that the smaller native earthworm *Allolobophora parva* accumulated significantly greater Pb concentrations than *E. fetida*. Subsequently, it was concluded that native earthworm species may exhibit a higher Pb accumulation potential as a result of increased tolerance to the heavy metal (Garg et al., 2009).

Earthworm activity can alter Pb bioavailability and subsequent uptake by earthworms themselves and other organisms. Sizmur and Hodson (2009) speculated that earthworms affect Pb mobility by modifying the availability of cations or anions. The concentration of water-soluble Pb was observed to increase following earthworm (*Lumbricus terrestris*) feeding activity in field-collected soils containing 132.7, 814.9, and 821.4 mg total Pb/kg (calculated BAFs of 0.27, 0.33, and 0.13, respectively) (Alonso-Azcarate et al., 2011). However, Coeurdassier et al. (2007) found that snails did not have a higher Pb content when earthworms were present, and that unexpectedly, Pb was higher in earthworm tissue when snails were present.

Despite significant Pb uptake by earthworms, Pb in earthworm tissue may not be bioavailable to predators. Pb in the earthworm (*Aporrectodea caliginosa*) was determined to be contained largely in the granular fraction (approximately 60% of total Pb), while the remaining Pb body burden was in the tissue, cell membrane, and intact cell fractions

(Vijver et al., 2006). However, this may vary by species, as (Li et al., 2008b) found that more than half of the Pb accumulated by *E. fetida* was contained within earthworm tissue and cell membranes. Regardless, Vijver et al. (2006) concluded that only a minority of earthworm-absorbed Pb would be toxicologically available to cause effects in the earthworms or in their predators.

Arthropods

Pb and other metals were analyzed in honeybees (*Apis mellifera*) foraging in sampling sites that included both urban areas and wildlife reserves in central Italy. (Perugini et al., 2011). Pb in whole bees ranged from 0.28 to 0.52 mg Pb/kg with the highest concentration in honeybees caught in hives near an airport and industrial area within the Rome metropolitan area. Cicadas pupating in historically Pb-arsenate-treated soils accumulated Pb at concentrations similar to those reported previously for earthworms (Robinson et al., 2007). Likewise, tissue Pb levels measured in Coleoptera specimens collected from areas containing average soil concentration of 45 and 71 mg Pb/kg exhibited a positive relationship with soil Pb content, although abundance was unaffected (Schipper et al., 2008). By contrast, two grasshopper species inhabiting Pb and Cd-contaminated areas near Zn smelting facilities exhibited different Pb accumulation rates. Locust (*Locusta migratoria*) collected from areas with an average Pb soil concentration of 540mg Pb/kg contained 47 mg Pb/kg, while grasshoppers (*Acrida chinensis*) inhabiting the same area accumulated 93.9 mg Pb/kg (Zhang et al., 2012). This gives respective BAFs of 0.09 and 0.17. Similarly, the Pb sequestration rates that were observed in two woodlouse species, *O. asellus* and *P. scaber*, were species-dependent (Gál et al., 2008). Both species were field collected at Pb-contaminated sites (average concentration, 245 mg Pb/kg dry weight; range, 21-638 mg Pb/kg dry weight), with *O. asellus* Pb levels averaging 43 mg Pb/kg over all sites, while *P. scaber* contained no detectable Pb residues. Pb concentration measured in granivorous rough harvester ants (*Pogonomyrmex rugosus*), in the seeds of some plant species they consume, and in surface soil, were all shown to decline with increasing distance from a former Pb smelter near El Paso, Texas, where soil leachable Pb at the three sites of ant collection ranged from 0.003 to 0.117 mg Pb/kg (Del Toro et al., 2010). Ants accumulated approximately twice as much Pb as was measured in seeds, but the study did not separate the effects of dietary exposure from those of direct contact with soil or respiratory intake.

6.3.3.3 Terrestrial Vertebrates

At the time of the 1977 Pb AQCD few studies of Pb exposure and effects in wild animals other than birds had been conducted. A limited number of rodent trapping studies near roadsides indicated general trends of species differences in Pb uptake and higher concentrations of Pb in habitats adjacent to high-traffic areas (U.S. EPA, 1977). In the 1986 Pb AQCD concentration of Pb in bone tissue was reported for selected herbivore, omnivore, and carnivore species [Table 8-2 in (U.S. EPA, 1986b)].

Tissue Pb residues in birds and mammals associated with adverse toxicological effects were presented in the 2006 Pb AQCD. In general, avian blood, liver, and kidney Pb concentrations of 0.2-3 µg Pb/dL, 2-6 mg Pb/kg wet weight, and 2-20 mg Pb/kg wet weight, respectively, were linked to adverse effects. A few additional studies of Pb uptake and tissue residues in birds and mammals conducted since 2006 are reviewed here.

In a study of blood Pb levels in wild Steller's eiders (*Polysticta stelleri*) and black scoters (*Melanitta nigra*) in Alaska, the authors compiled avian blood Pb data from available literature to develop reference values for sea ducks (Brown et al., 2006). The background exposure reference value of blood Pb was <20 µg Pb/dL, with levels between 20 and 59 µg Pb/dL as indicative of Pb exposure. Clinical toxicity was in the range of 60-99 µg Pb/dL in birds while >100 µg Pb/dL results in acute, severe toxicity. In measurement of blood Pb with a portable blood Pb analyzer, only 3% of birds had values indicating exposure and none of the birds had higher blood Pb levels or clinical signs of toxicity. Tissue distribution of Pb in liver, kidney, ovary and testes of rain quail (*Coturnix coramandelica*) following oral dosing of 0.5 mg Pb/kg, 1.25 mg Pb/kg or 2.5 mg Pb/kg Pb acetate for 21 days indicated that Pb uptake was highest in liver and kidney and low in ovary and testes (Mehrotra et al., 2008). Resident feral pigeons (*Columba livia*) captured in the urban and industrial areas of Korea exhibited increased lung Pb concentration, ranging from 1.6 to 1.9 mg Pb/kg wet weight (Nam and Lee, 2006). However, tissue concentration did not correlate with atmospheric Pb concentration, so the authors concluded that ingestion of particulate Pb (paint chips, cement, etc.) in the urban and industrial areas was responsible for the pigeons' body burden. Similarly, 70% of American woodcock (*Scolopax minor*) chicks and 43% of American woodcock young-of-year collected in Wisconsin, U.S., exhibited high bone Pb levels of 9.6-93 mg Pb/kg dry weight and 1.5-220 mg Pb/kg, respectively, even though radiographs of birds' gastrointestinal tracts revealed no evidence of shot ingestion (Strom et al., 2005). Authors hypothesized that unidentified anthropogenic sources may have caused the observed elevated Pb levels.

In addition to birds, soil-dwelling mammals can also bioaccumulate atmospherically-sourced Pb. Northern pocket gophers (*Thomomys talpoides*) trapped within the Anaconda Smelter Superfund Site were shown to accumulate atmospherically deposited Pb. Gopher liver and carcass Pb concentration averaged 0.3 and 0.4 mg Pb/kg wet weight on low Pb soils (47 mg Pb/kg), 0.4 and 0.9 mg Pb/kg wet weight in medium Pb soils (95 mg Pb/kg) and 1.6 and 3.8 mg Pb/kg wet weight in high Pb soils (776.5 mg Pb/kg) (Reynolds et al., 2006). Likewise, rats trapped in the vicinity of a Kabwe, Zambia Pb-Zn mine had significantly elevated liver and kidney Pb concentrations. Soil Pb concentrations were measured between 9 and 51,188 mg Pb/kg (approximate average of 200 mg Pb/kg dry weight), while rat liver and kidney Pb concentrations ranged between 0.009 and 7.3 mg Pb/kg dry weight and 0.3 and 22.1 mg Pb/kg dry weight, respectively. Consequently, residence in the mining region was correlated to significantly increased Pb body burdens for rats (Nakayama et al., 2011). Angelova et al. (2010) reared rabbits on a fodder mixture containing Pb-contaminated rapeseed (grown adjacent to a metal works plant). Following a four-week exposure, Pb was most heavily concentrated in rabbit kidney tissue (3.9 mg Pb/kg and 1.9 mg Pb/kg, for high and low diet respectively), bone (1.0 and 0.3 mg Pb/kg, respectively), and liver (0.6 and 0.4 mg Pb/kg, respectively). Yucatan micropigs (*Sus scrofa*) and Sprague-Dawley rats (*Rattus norvegicus*) reared on Pb-contaminated soil (5% of 1,000 mg Pb/kg soil as dietary component) consumed significantly different amounts of Pb. Over a 30-day period, rats consumed an average of 19.4 mg Pb, while micropig intake averaged 948 mg Pb (Smith et al., 2009a). This resulted in significantly higher Pb accumulation in micropigs, based on liver, blood, kidney and bone Pb concentrations (average concentrations of 1.2, 25, 0.9, and 9 mg Pb/kg for micropigs, and 0.2, 7, 0.5, and 1.5 mg Pb/kg for rats, respectively).

Casteel et al. (2006) found that bioavailability of Pb from environmental soil samples in swine (*Sus domestica*) depended on Pb form or type, with high absorption of Pb from cerussite and Mn-Pb oxides and poor absorption of Pb from galena and anglesite. Juvenile swine (approximately 5-6 weeks old and weighing 8-11 kg) were fed Pb-contaminated soils collected from multiple sources for 15 days (concentration range of 1,270 to 14,200 mg Pb/kg) to determine the relative bioavailability. While Pb concentrations were roughly equivalent in blood, liver, kidney, and bone tissues, individual swine exhibited different uptake abilities (Casteel et al., 2006).

Consistent with observations in humans, dietary Ca^{2+} deficiency (0.45 mg Ca^{2+} daily versus 4 mg under normal conditions) was linked to increased accumulation of Pb in zebra finches (*Taeniopygia guttata*) that were provided with drinking water containing 20 mg Pb/L (Dauwe et al., 2006). Liver and bone Pb concentration were increased by an approximate factor of three, while Pb concentration in kidney, muscle, and brain tissues

were roughly doubled by a Ca^{2+}-deficient diet. However, it is not known whether this level of dietary Ca^{2+} deficiency is common in wild populations of birds.

6.3.3.4 Food Web

In addition to the organism-level factors reviewed above, understanding the bioavailability of Pb along a simple food chain is essential for determining risk to terrestrial animals. While the bioavailability of ingested soil or particles is relatively simple to measure and model, the bioavailability to secondary consumers of Pb ingested and sequestered by primary producers and primary consumers is more complex. Kaufman et al. (2007) caution that the use of total Pb concentration in risk assessments can result in overestimation of risk to ecological receptors, and they suggest that the bioaccessible fraction may provide a more realistic approximation of receptor exposure and effects. This section reviews recent literature that estimates the bioaccessible fraction of Pb in dietary items of higher order consumers, and various studies suggesting that Pb may be transferred through the food chain but that trophic transfer of Pb results in gradual attenuation, i.e., lower concentration at each successive trophic level.

Earthworm and plant vegetative tissue collected from a rifle and pistol range that contained average soil Pb concentration of 5,044 mg Pb/kg were analyzed for Pb content and used to model secondary bioavailability to mammals (Kaufman et al., 2007). Earthworms were determined to contain an average of 727 mg Pb/kg, and the Pb content of unwashed leaf tissues averaged 2,945 mg Pb/kg. Canonical correspondence analysis detected no relationship between earthworm and soil Pb concentration, but did show correlation between unwashed vegetation and soil concentration. The authors noted that the relatively high Pb concentration of unwashed as opposed to washed vegetation indicated the potential importance of aerial deposition (or dust resuspension) in determining total vegetative Pb concentration. Based on the mammalian gastric model, they noted that 50% of vegetation tissue Pb and 77% of earthworm tissue Pb was expected to be bioavailable to consumers. The avian gizzard model indicated that 53% of soil Pb and 73% of earthworm Pb was bioaccessible to birds; and, for both mammals and birds, the bioaccessible fraction of Pb was a function of total Pb concentration.

The transfer of Pb from soils contaminated by a Pb-Zn mine was limited along a soil-plant-insect-chicken food chain (Zhuang et al., 2009). In soils averaging 991 mg Pb/kg, plants of the fodder plant *Rumex patientia X tianschanicus* sequestered an average of 1.6 mg Pb/kg wet weight in the shoot tissue, while larvae of the leafworm *Spodoptera litura* accumulated an average Pb concentration of 3.3 mg Pb/kg wet weight *S. litura*-fed chickens (*Gallus gallus domesticus*) accumulated 0.58 mg Pb/kg and 3.6 mg Pb/kg in

muscle and liver tissue, respectively, but only liver Pb burden was increased significantly relative to controls. A large proportion of ingested Pb was excreted with the feces. Likewise, a wading bird species, the black-tailed godwit (*Limosa limosa*) was shown to accumulate Pb from earthworms residing in Pb-contaminated soils (Roodbergen et al., 2008). Pb concentration in eggs and feathers was increased in areas with high soil and earthworm Pb concentration (336 and 34 mg Pb/kg, respectively): egg Pb concentration averaged 0.17 mg Pb/kg and feather concentration averaged 2.8 mg Pb/kg. This suggests that despite a residence breeding time of only a few months, this bird species could accumulate Pb when breeding areas are contaminated.

Rogival et al. (2007) showed significant positive correlations between soil Pb concentration along a gradient (approximately 50 to 275 mg Pb/kg) at a metallurgical plant, and Pb concentration in both acorns (from *Quercus robur*) and earthworms (primarily *Dendrodrilus rubidus* and *Lumbricus rubellus*) collected on site. Acorn and earthworm Pb contents were, in turn, positively correlated with the Pb concentration in the liver, kidney, and bone tissues of locally trapped wood mice (*Apodemus sylvaticus*).

The uptake and transfer of Pb from soil to native plants and to red deer (*Cervus elaphus*) was investigated in mining areas of the Sierra Madrona Mountains in Spain (Reglero et al., 2008). The authors reported a clear pattern between plant Pb concentration and the Pb content of red deer tissues with attenuation (i.e., decreasing concentration) of Pb up the food chain. Interestingly, soil geochemistry likely was affected by mining activity as Holm oak (*Quercus ilex*), gum rockrose (*Cistus ladanifer*), elm leaf blackberry (*Rubus ulmifolius*), and grass (Graminae) tissues collected from mining areas exhibited increased Pb levels (up to 98 mg Pb/kg in grasses and 21 mg Pb/kg in oak) despite the fact that total soil Pb concentrations were not significantly greater than those of the non-mining areas.

Positive relationships were observed between *Cepaea nemoralis* snail tissue Pb levels and Pb concentration measured in *Urtica dioica* leaves in field-collected samples from areas characterized by metal soil contamination (approximately 200 to 400 mg Pb/kg) (Notten et al., 2005). Inouye et al. (2007) found that several invertebrate prey of fence lizards, including *Acheta domestica* crickets, *Tenebrio molitor* beetles, and *P. scaber* isopods, accumulate Pb from dietary exposures (10, 50, 100, 250, 500, 750, and 1,000 mg Pb/kg) lasting between 44 and 72 days. By day 44, Pb body burdens of crickets were 31, 50 and 68 mg Pb/kg (wet weight) at the three highest dietary exposures, respectively. Isopods and beetle larvae accumulated significantly less Pb, with average body burdens of 10, 15, and 14 mg Pb/kg following 56 days of exposure; and 12, 14, and 31 mg Pb/kg following 77 days of exposure, respectively. For all invertebrates tested, Pb was sequestered partly in the exoskeleton, and partly in granules. Exoskeleton Pb may be

available to predators, but returns to background level with each shedding, while granular Pb is likely unavailable, at least to other invertebrates (Vijver et al., 2004).

In a comparison of rural and urban blackbirds (*Turdus merula*), Sheifler et al.(2006a) found that while Pb concentration in unwashed tail feathers was equivalent in both populations, urban birds had higher tissue concentrations. Pb content of urban earthworms was also higher than that of rural earthworms. Hypothesizing that tail feather Pb reflected deposition from air and resuspended dust, the authors suggested that elevated Pb in the urban birds was mostly dietary in origin.

Overall, studies of Pb transfer in food webs have established the existence of pervasive trophic transfer of the metal, but no consistent evidence of trophic magnification. It appears that on the contrary, attenuation is common as Pb is transferred to higher trophic levels. However, many individual transfer steps, as from particular plants to particular invertebrates, result in concentration, which may then be undone when stepping to the next trophic level. It is possible that whether trophic transfer is magnifying or attenuating depends on Pb concentration itself. Kaufman et al. (2007) determined that, at low concentrations of soil Pb, risk to secondary consumers (birds and mammals) was driven by the bioavailability of Pb in worm tissues, while at high soil concentrations, bioavailability of soil-associated Pb was more critical. The authors concluded that incorporation of bioavailability/bioaccessibility measurements in terrestrial risk assessments could lead to more accurate estimates of critical Pb levels in soil and biota. Finally, while trophic magnification does greatly increase exposure of organisms at the higher levels of the food web, these studies establish that atmospherically deposited Pb reaches species that have little direct exposure to it. For those species, detrimental effects are not a function of whether they accumulate more Pb than the species they consume, but of the absolute amount they are exposed to, and their sensitivity to it.

6.3.4 Biological Effects of Pb in Terrestrial Systems

Various effects can be observed in exposed terrestrial species following uptake and accumulation of Pb. While many of the responses are specific to organism type, induction of antioxidant activities in response to Pb exposure has been reported in plants, invertebrates, and vertebrates. Observed biological effects caused by exposure to atmosphere-derived Pb are discussed in this section, while the results of dose-response experimentation are reported in Section 6.3.5. Because environmental releases of Pb often include simultaneous release of other metals, it can be difficult to identify Pb-specific effects in field studies, with the exception of effects from leaded gasoline and some Pb smelter deposition. Many laboratory studies that expose organisms to natural

soils (or to biosolids-amended soils) also include exposure to multiple metals. There is some information about mechanisms of metal interactions, such as through competition for binding locations on specific enzymes or on cellular receptors, but generally such interactions (particularly of multiple metals) are not well understood (ATSDR, 2004). Despite a few well-known examples of metal antagonism (e.g., Cu and Mo or Cd and Zn), it is common practice to assume additivity of effects (Fairbrother et al., 2007). Because this review is focused on effects of Pb, studies reviewed for this section and the following include only those for which Pb was the only (or primary) metal to which the organism was exposed. All reported values are from exposures in which concentrations of Pb were analytically verified unless nominal concentrations are stated.

6.3.4.1 Terrestrial Plants and Lichen

Pb exposure has been linked to decreased photosynthesis in affected plants, significant induction of antioxidant activities, genetic abnormalities, and decreased growth. Induction of antioxidant responses in plants has been shown to increase tolerance to metal soil contamination, but at sufficiently high levels, antioxidant capacity is exceeded, and metal exposure causes peroxidation of lipids and DNA damage, eventually leading to accelerated senescence and potentially death (Stobrawa and Lorenc-Plucinska, 2008).

Effects on Photosystem and Chlorophyll

Photosynthesis and mitosis were recognized as targets of Pb toxicity in plants in the 1977 Pb AQCD and additional effects of Pb on these processes were reported in subsequent Pb AQCDs (U.S. EPA, 2006c, 1986b, 1977). The effect of Pb exposure on the structure and function of plant photosystem II was recently studied in giant duckweed, *Spirodela polyrrhiza* (Ling and Hong, 2009). Although this is an aquatic plant, photosystem II is present in all plants. This finding thus provides support for effects on photosystem II being the cellular-level mechanism that leads to decreases photosynthesis observed in other plants. The Pb concentration of extracted photosystem II particles was found to increase with increasing environmental Pb concentration, and increased Pb concentration was shown to decrease emission peak intensity at 340 nm, amino acid excitation peaks at 230 nm, tyrosine residues, and absorption intensities. This results in decreased efficiency of visible light absorption by affected plants. The authors theorized that Pb^{2+} may replace either Mg^{2+} or Ca^{2+} in chlorophyll or the oxygen-evolving center, inhibiting photosystem II function through an alteration of chlorophyll structure. Consistently with these results, Wu et al. (2008b) demonstrated that Pb exposure interfered with and decreased light absorption by spinach (*Spinacia oleracea*) plants. Spinach seeds were soaked in 5, 12, or

25 mM Pb chloride (1036, 2486, or 5180 mg Pb/L) for 48 hours prior to germination, and following 42 days of growth, plants were sprayed with Pb chloride solutions. Chloroplast absorption peak intensity, fluorescence quantum yield at 680 nm, and whole-chain electron transport rate all decreased with Pb exposure, as did photosystem II photoreduction and oxygen evolution. Similarly, the photosynthetic rate of maize (*Zea mays*) seedlings decreased over 21 days exposure to Pb, and measured leaf Pb concentrations in photosynthetically-depressed seedlings ranged from approximately 100 to 300 mg Pb/kg dry weight (Ahmad et al., 2011). Liu et al. (2010a) observed that chlorophyll *a* and *b* content in wheat grown in soils spiked with Pb nitrate rose with length of exposure until 14 days, at which point chlorophyll decreased. At nominal exposures of 0.1 and 0.5 mM Pb (20.72 and 103.6 mg Pb/L) in hydroponic solution for 50 days, concentration of chlorophyll *a* and b was decreased in radish (*R. sativus)* (Kumar and Tripathi, 2008). Changes in chlorophyll content in response to Pb were also observed in lichen and moss species following exposures intended to simulate atmospheric deposition (Carreras and Pignata, 2007). *Usnae amblyoclad*a lichen was exposed to aqueous Pb solutions of 0.5, 1, 5, and 10 mM Pb nitrate (103.6, 207.2, 1,036, and 2,072 mg Pb/L); chlorophyll *a* concentration was shown to decrease with increasing Pb exposure. However, the ratio of lichen dry weight to fresh weight increased following Pb exposures. It should be noted that highly productive *Sphagnum* mosses accumulated atmospheric Pb at the same rate as slower growing mosses, indicating that moss growth allowed for further Pb uptake, rather than a "dilution" effect (Kempter et al., 2010). As compared to other metals, however, Pb caused less physiological damage, which the authors attributed to the metal's high affinity for binding to and sequestration within cell walls (Carreras and Pignata, 2007).

The effect of Pb exposure on chlorophyll content of the moss and liverwort species *Thuidium delicatulum*, *T. sparsifolium*, and *Ptychanthus striatus* was investigated following immersion in six solutions of Pb nitrate containing from 10^{-10} to 10^{-2} M Pb (0.00002 to 2,072 mg Pb/L) (Shakya et al., 2008). Both chlorophyll *a* and total chlorophyll content of the mosses (*T. delicatulum* and *T. sparsifolium*) decreased with increasing Pb exposure. For the liverwort, increasing Pb exposure resulted in decreases in content of chlorophyll *a*, chlorophyll *b*, and total chlorophyll. Further, the total chlorophyll content of *Hypnum plumaeforme* mosses was decreased by 5.8% following exposure to the highest concentration, while lower exposures slightly elevated chlorophyll content.

These studies suggest that exposure to Pb has an impact on photosynthetic pigments, but the exposure methods (seed soaking, spraying of Pb chloride solutions, hydroponic growth systems) make it difficult to compare these effects to what might occur under the uncontrolled conditions encountered in natural environments. These experiments bring to

light the presence of effects, and the underlying mechanisms, but strong uncertainties remain regarding the natural concentrations at which these effects would be observed.

Response of Antioxidants

Increased antioxidant activity is a common response to Pb exposure, although this endpoint may not necessarily be an indication of deleterious effects on plant vitality. Increases in reactive oxygen species with increasing exposure to Pb from 20 mg Pb/kg soil to 2,000 mg Pb/kg have been demonstrated in broad bean (*Vicia faba*) (Wang et al., 2010c; Wang et al., 2010a; Wang et al., 2008b) and tomato (*Lycopersicon esculentum*) (Wang et al., 2008a), where they were accompanied up to approximately 500mg Pb/kg by proportional increases in superoxide dismutase (SOD), glutathione, guaiacol peroxidase, and lipid peroxidation, as well as decreases in catalase. Spinach seedlings grown in soil containing six increasing concentrations of Pb from 20 to 520 mg Pb/kg exhibited higher production of reactive oxygen species, increased rates of lipid peroxidation and increased SOD concentrations. Many of these responses persisted for 50 days after germination and growth in the Pb-contaminated soil (Wang et al., 2011a). Similarly, the bryophyte mosses *Hypnum plumaeforme*, *Thuidium cymbifolium*, and *Brachythecium piligerum* exposed to Pb solutions of greater than 0.1 mM Pb for 48 hours exhibited increased production of O_2^- radical and H_2O_2, although no single moss species could be identified as most sensitive to Pb exposure (Sun et al., 2011). Increased rates of lipid peroxidation were also observed in Pb-exposed mosses; however, SOD and catalase activity was suppressed or unaffected by Pb.

Reddy et al. (2005) found that horsegram (*Macrotyloma uniflorum*) and bengalgram (*Cicer arietinum*) plants watered with Pb solutions containing 200, 500, and 800 mg Pb/L exhibited increased antioxidant activity: at exposures of 800 mg Pb/L, root and shoot SOD activity increased to 2–3 times that of controls, and induction was slightly higher in *M. uniflorum*. Similarly, catalase, peroxidase, and glutathione-S-transferase activities were elevated in Pb-stressed plants, but were again higher for *M. uniflorum*. Antioxidant activities were also markedly greater in the root tissues than the shoot tissues of the two plants, and were very likely related to the higher Pb accumulation rate of the roots. The effectiveness of the up-regulation of antioxidant systems in preventing damage from Pb uptake was evidenced by lower malondialdehyde (MDA) (a chemical marker of lipid peroxidation) concentration in *M. uniflorum* versus *C. arietinum*, indicating a lower rate of lipid peroxidation in response to *M. uniflorum's* higher antioxidant activity.

Gupta et al. (2010) contrasted responses of two ecotypes of *Sedum alfredii* (an Asian perennial herb), one an accumulator of Pb collected from a Pb and Zn mining area, and the other not. Glutathione level was increased in both, and root and shoot lengths were

decreased following long-term exposures to Pb up to 200 µM (41.4 mg Pb/L) in hydroponic solution. However, the accumulator plants exhibited greater SOD and ascorbate peroxidase activity, likely as a result of greater Pb uptake and a concurrent increased detoxification capacity. Similar results were reported by Islam et al. (2008): following Pb exposures of 200 µM (41.4 mg Pb/L), catalase, ascorbic acid, and glutathione levels of another Chinese herb, *Elsholtzia argyi*, were increased, while SOD and guaiacol peroxidase activities decreased. Microscopic analysis also showed that affected plants exhibited abnormal chloroplast structures. The response of glutathione was further confirmed in wheat (Liu et al., 2010a) grown in soils spiked with Pb nitrate. Evidence of increasing lipid peroxidation (MDA accumulation) with increasing Pb exposure was also found in mosses (Sun et al., 2009) and lichens. Lichens field-collected from the trunks of poplar (*Populus tremula*) trees in eastern Slovakia were chemically analyzed for metal concentration arising from exposure to smelter pollution (Dzubaj et al., 2008). These concentrations (ranging from 13 to 1,523 mg Pb/kg dry weight) were assessed in relation to physiological variables, including chlorophyll *a* and *b*, carotenoids, photosystem II activity, CO_2 gas exchange (respiration), and MDA content. Lichen Pb levels were significantly correlated only with MDA content. Determination of plant chitinase content following exposure to As, Cd and Pb indicated that while levels of these defense proteins were elevated by As and Cd, chitinase levels were not increased following exposure to Pb (Békésiová et al., 2008). As in studies of effects on photosynthesis, the methods used for exposure make it difficult to compare these effects to what might occur under the uncontrolled conditions encountered in natural environments.

Growth

Evidence of effects of Pb on higher growth processes in terrestrial plants was reported in early NAAQS reviews. Impacts to growth can lead to effects at the population-level of biological organization and higher (Section 6.1.1). Growth effects of Pb on plants in the 1977 Pb AQCD primarily included visible growth responses observed in laboratory studies with plants grown in artificial nutrient culture (U.S. EPA, 1977). No Pb toxicity was observed in plants growing under field conditions at the time of the 1977 Pb AQCD. Indirect effects of Pb on plant growth (i.e., inhibition of uptake of other nutrients when Pb is present in the plant) were also reported in the 1977 Pb AQCD. In the 1986 Pb AQCD mechanisms of Pb effects on growth included reduction of photosynthetic rate, inhibition of respiration, cell elongation, root development, or premature senescence (U.S. EPA, 1986b). In 1986, EPA reported that all of these effects were observed to occur in isolated cells or in plants grown hydroponically in solutions comparable to 1 to 2 mg Pb/kg soil or in soils with 10,000 mg Pb/kg or greater (U.S. EPA, 1986b). Pb effects on

other plant processes, especially maintenance, flowering, and hormone development had not been studied at the time of the 1986 Pb AQCD and remain poorly characterized.

Recent evidence for growth effects in terrestrial plants available since the 2006 Pb AQCD is reviewed below and summarized in Table 6-4. Both growth and carotenoid and chlorophyll content of *Brassica juncea* (mustard) plants were negatively affected by Pb exposure (John et al., 2009). Nominal Pb treatments of 1,500 µM (311 mg Pb/L) as Pb acetate solution decreased root lengths and stem heights by 50% after 60 days. Exposure to 600 µM Pb (124 mg Pb/L) and greater, decreased carotenoid content, while chlorophyll *a* was decreased at Pb exposures of 450 µM (93 mg Pb/L) and higher. However, when smelter ash–spiked soils containing 1,466 mg Pb/kg (and 18.6 mg Cd/kg) or 7,331 mg Pb/kg (98.0 mg/kg Cd) were used to grow maize (*Zea mays*), as well as other metals in high concentrations, effects were seen in growth or chlorophyll production only at the higher concentration (Komarek et al., 2009). Given the low solubility of smelter ash, these observations are consistent with solubility being a key determinant of bioavailability. Similarly, wheat seedling growth was unaffected when exposed to soil leachate containing up to 0.7 mg Pb/L for six weeks. Lettuce seedling root growth was negatively correlated to leachate Pb concentration, but this correlation was only significant for week 3 and week 6 measurements. Authors concluded that although the total concentrations of multiple metals in tested soils and leachates exceeded Canadian Environmental Quality Guidelines, no toxic or only slightly toxic effects occurred following exposure to the metal mixture (Chapman et al., 2010).

Chinese cabbage (*Brassica pekinensis*) exposed to Pb-containing soils exhibited depressed nitrogen assimilation as measured by shoot nitrite content, nitrate reductase activity, and free amino acid concentration (Xiong et al., 2006). The authors planted germinated cabbage seeds in soils spiked with Pb acetate to give final soil concentrations of 0.2 (control), 4, and 8 mmol Pb/kg dry weight total Pb (41, 829, and 1,658 mg Pb/kg) and collected leaf samples for 11 days. At exposures of 4 and 8 mmol Pb/kg (828.8 and 1,657.6 mg Pb/kg), leaf nitrate content was decreased by 29% and 20% relative to control. Free amino acid content in exposed plants was 81% and 82% of control levels, respectively. *B. pekinensis* shoot biomass was observed to decrease with increasing Pb exposures, with biomass at the two highest Pb exposures representing 91% and 84% of control growth, respectively.

Nitrogen, potassium, and phosphorus concentrations in the shoot and root tissues of four canola cultivars (*Brassica napus*) also decreased as spiked soil Pb concentrations increased from 0 to 90 mg/kg. At the highest soil Pb concentration, nitrogen concentrations were reduced 56% in roots and 58% in shoots versus control levels, while phosphorous concentrations were reduced 37% and 45%, respectively, and potassium

content decreased by 42% in both tissues (Ashraf et al., 2011). Cultivation in Pb-spiked soils was also linked to decreased shoot and root biomass (32% and 62%, respectively at 90 mg Pb/kg).

Genetic and Reproductive Effects

Exposure to Pb also resulted in genetic abnormalities, including bridges, condensed bivalents, and laggards, in the meiotic cells of pea plants (*Lathyrus sativus*) (Kumar and Tripathi, 2008). Seeds were germinated in soils amended with Pb nitrate at concentrations of 25, 50, 100, 200, and 300 mg Pb/kg, and concentrations of 100 mg Pb/kg and greater were found to be genotoxic or detrimental to pea viability. Cenkci et al. (2010) exposed fodder turnip (*B. rapa*) to 0.5 to 5 mM of Pb nitrate (103.6 to 1036 mg Pb/L) for 6 days and showed decreased genetic template stability (as quantified by random amplified polymorphic DNA profiles) and decreased photosynthetic pigments.

Two genotypes of maize seedlings exhibited a significant and concentration-dependent reduction in seed germination following 7 days of Pb treatment in nutrient solution of 0.01, 0.1 and 1 mg Pb/L as Pb sulfate (Ahmad et al., 2011). Pb exposure also decreased germination rate and growth, and increased pollen sterility in radish grown for 50 days in hydroponic solutions containing 0.5 mM Pb (104 mg Pb/L) (Kumar and Tripathi, 2008). Plants exposed to Pb exhibited decreased growth, curling and chlorosis of young leaves, and decreased root growth. In addition, Gopal and Rizvi (2008) showed that Pb exposure increased uptake of phosphorus (P) and iron (Fe) and decreased sulfur (S) concentration in radish tops.

Interestingly, as in zebra finch (Section 6.3.3.3) Ca^{2+} was found to moderate the effects of Pb in both monocotyledon and dicotyledon plant seedlings, with tomato (*Lycopersicon esculentum*), rye (Lolium sp.), mustard, and maize plants exhibiting increased tolerance to Pb exposures of 5, 10, and 20 mg Pb/L in the presence of Ca^{2+} concentration of 1.2 mM (249 mg Pb/L) and higher (Antosiewicz, 2005).

6.3.4.2 Terrestrial Invertebrates

Exposure to Pb also causes antioxidant effects, reductions in survival and growth, as well as decreased fecundity in terrestrial invertebrates as summarized in the 2006 Pb AQCD (U.S. EPA, 2006b). Alterations in reproduction, growth and survival at the species level can lead to effects at the population-level of biological organization and higher (Section 6.1.1). In addition to these endpoints, recent literature also indicates that Pb exposure can cause significant neurobehavioral aberrations, and in some cases,

endocrine-level impacts. Second-generation effects have been observed in some invertebrate species.

The morphology of γ-aminobutyric acid (GABA) motor neurons in *Caenorhabditis elegans* nematodes was affected following exposure to Pb nitrate for 24 hours (Du and Wang, 2009). The authors determined that exposures as low as 2.5 µM Pb nitrate (0.5 mg Pb/L) could cause moderate axonal discontinuities, and observed a significant increase in the number of formed gaps and ventral cord gaps at Pb nitrate exposures of 75 and 200 µM (6 and 41 mg Pb/L). Younger *C. elegans* larvae were more likely to exhibit neurobehavioral toxicity symptoms in response to Pb exposure at 2.5 µM (0.5 mg Pb/L) (Xing et al., 2009b). Neural degeneration, as demonstrated by dorsal and ventral cord gaps and neuronal loss was also more pronounced in young larval *C. elegans* than in older larvae and adults (Xing et al., 2009c). *C. elegans* nematodes exposed to Pb concentration as low as 2.5 µM (0.5 mg Pb/L) for 24 hours also exhibited significantly altered behavior characterized by decreased head thrashes and body bends. Exposures of 50 µM Pb (10 mg Pb/L) and greater decreased the number of nematode forward turns (Wang and Xing, 2008). Chemotaxis toward NaCl, cAMP, and biotin was also decreased in *C. elegans* nematodes exposed to Pb concentration greater than 2.5 µM (0.5 mg Pb/L) (Xing et al., 2009a). This evidence suggests that Pb may exert neurotoxic action in invertebrates as it does in vertebrates. However, it is unclear how these behavioral aberrations would affect fitness or survival (Wang and Xing, 2008).

In a study of *C. elegans* exposed to 4 sub-lethal concentrations of Pb nitrate between 25 and 100 µM (5 and 21 mg Pb/L), Vigneshkumar et al. (2013) observed upregulation of both catalase and antimicrobial response-related genes. When challenged with addition of a pathogenic strain of *Pseudomonas aeruginosa*, exposed *C. elegans* showed greater resistance to microbial colonization than controls.

Younger individuals also appear to be more sensitive to the reproductive effects of Pb exposure. Guo et al. (2009) showed that concentrations of 2.5, 50, and 100 µM Pb (0.5, 10, and 21 mg Pb/L) had greater significant adverse effects on reproductive output when early-stage larval *C. elegans* were exposed. Adult *C. elegans* exhibited decreased brood size only when exposed to the highest Pb concentration.

The progeny of *C. elegans* nematodes exposed nominally to 2.5, 75, and 200 µM Pb nitrate (0.5, 16, and 41 mg Pb/L) exhibited significant indications of multi-generational toxicity (Wang and Yang, 2007). Life spans of offspring were decreased by increasing parental Pb exposure, and were comparable to the reductions in parental life-spans. Similarly, diminished fecundity was observed in the progeny of *C. elegans* exposed to Pb (9%, 19%, and 31% reductions of control fecundity, respectively), although the decrease was smaller than in the exposed parental generation (reductions of

52%, 58%, and 65%, respectively). Significant behavioral defects affecting locomotion were also observed in the offspring, but these impacts were not determined to be concentration-dependent. Reproductive effects of Pb exposure were also observed in springtails *F. candida* following 10-day exposure to Pb-spiked soils. Egg hatch significantly decreased at nominal concentrations of 1,600 mg Pb/kg dry soil and higher and the EC_{50} for hatching was 2,361 mg Pb/kg dry soils (Xu et al., 2009b).

E. andrei earthworms exposed to 21 different soils, each containing 2,000 mg Pb/kg freshly added Pb, for 28 days exhibited highly variable mortality, ranging from 0% to 100%, (Bradham et al., 2006). Pb body burden of exposed worms ranged from 29 to 782 mg Pb/kg. Internal Pb concentration was also negatively correlated to reproductive output. CEC and pH were found to be the principal soil characteristics determining the differences in those effects, although the apparent role of CEC may only have been due to its correlation with other soil characteristics. Low soil Pb concentration (5 mg Pb/kg) also decreased the protein content of *E. fetida* earthworms during a 7-day exposure (Li et al., 2009b). Higher Pb concentration had no effect on protein production. However, cellulase activity was increased by the 7-day exposures to Pb at all exposure concentrations (31%, 13%, and 23% of control activity at exposures of 5, 50, and 500 mg Pb/kg, respectively), which the authors reported as an indication of detrimental effects on worm metabolism. By contrast, Svendsen et al. (2007) found that *L. rubellus* earthworms exposed for 42 days to field-collected smelter-polluted soils containing average Pb concentration of 106, 309, and 514 mg Pb/kg dry weight exhibited normal survival and cocoon production rates, even though they accumulated more Pb with increased environmental concentration. The much smaller effect may be explained by the increased aging time undergone by field soil, causing a larger fraction of the total Pb to be complexed and sequestered by organic and inorganic compounds. Similarly, earthworms (*E. fetida*) exposed to field-collected soils with concentrations of Pb and As up to 390 mg/kg and 128 mg/kg, respectively, due to historical treatments of Pb-arsenate pesticides, exhibited no change in survival, behavior or morphology (Delistraty and Yokel, 2011). Soil aging (e.g., from of the time of Pb-arsenate applications in 1942 to soil collection in approximately 2009) likely reduced Pb bioavailability to earthworms.

As in plants, induction of metal chelating proteins and antioxidant activity in invertebrates is affected by exposure to Pb. Metallothionein production in earthworms (*Lampito mauritii*) was significantly induced following exposure to Pb-contaminated soil. Tissue metallothionein levels increased after a two-week exposure to 75 to 300 mg Pb/kg soil, although by 28 days levels had begun to decrease, perhaps as a result of Pb toxicity (Maity et al., 2011). Further, the induction of antioxidant activity was correlated to standard toxicity measurements in *Theba pisana* snails (Radwan et al., 2010). Topical application of Pb solutions (estimated to be 500 to 2,000 µg Pb per animal) to snails

resulted in decreased survival, increased catalase and glutathione peroxidase activities, and decreased glutathione concentration. The 48-hour LD_{50} concentration was determined to be 653 µg per snail, as measured in digestive gland tissue. Snail glutathione content was decreased at exposures of 72.2% of the 48-hour LD_{50} value, while Pb exposure at 40% of the 48-hour LD_{50} value induced catalase and glutathione peroxidase activities.

Dietary exposure to Pb also affected *T. pisana* snail growth. After three weeks on Pb-contaminated diet, snail feeding rates were depressed by all Pb exposures (nominal concentration of 50 to 15,000 mg Pb/kg diet dry weight) (El-Gendy et al., 2011). A five week dietary exposure to 1,000 mg Pb/kg and greater resulted in reduced snail growth. Food consumption, growth, and shell thickness were also observed to decrease with increasing diet Pb in juvenile *A. achatina* snails (7 levels between 0 and 1,344 mg Pb/kg feed, for 12 weeks) (Ebenso and Ologhobo, 2009a). A similar depression of growth was observed in sentinel juvenile *A. achatina* snails deployed at Pb-polluted sites in the Niger Delta region of Nigeria. Although snail mortality was not increased significantly by exposure to soil Pb up to 1,200 mg Pb/kg, a concentration-dependent relationship was established for growth, with significant reduction observed at 12-week exposures to 20 mg Pb/kg (Ebenso and Ologhobo, 2009b). However, consumption of field-collected Pb-polluted *U. dioica* leaves containing 3 mg Pb/kg stopped all reproductive output in *C. nemoralis*. Snails also exhibited diminished food consumption rates when offered leaves with both low (1.5 mg Pb/kg) and high Pb content, but the mechanism of the dietary aversion was not defined (Notten et al., 2006).

Chronic dietary exposure to Pb was also examined in post-embryonic oribatid mites (*Archegozetes longisetosus*) (Kohler et al., 2005). Both algae and bark samples were soaked in 100 mg/L Pb as Pb nitrate and provided as diet and substrate, respectively, to larval mites. In addition to elevated heat shock proteins (hsp70), 90.8% of the protonymphs exhibited significant leg deformities, including abnormal claws, shortened and thickened legs, and translocated setae. Although not specifically discussed, it is very likely that these deformities would decrease mite mobility, prey capture, and reproductive viability. While there is some evidence that oribatid mites exhibit Pb avoidance behavior, this response may not significantly reduce Pb exposure and effects. Although soil-inhabiting mites (*Oppia nitens*) were observed to avoid high Pb concentrations, the EC_{50} for this behavior was approximately five times higher than the chronic EC_{50} for reproduction (8,317 and 1,678 mg Pb/kg, respectively) (Owojori et al., 2011). Consequently, it is unlikely that oribatid mites will avoid soils containing toxic Pb concentrations.

Lock et al. (2006) compared the toxicity of both laboratory-spiked soils and field-collected Pb-contaminated soils to springtails (*F. candida*). The 28-day EC_{50} values derived for *F. candida* ranged from 2,060 to 3,210 mg Pb/kg in leached and unleached Pb-spiked soils, respectively, whereas field-collected soils had no significant effect on springtail reproduction up to (but not including) 14,436 mg Pb/kg (Lock et al., 2006). Consequently, leaching soils prior to use in bioassays had only a slight effect on Pb toxicity to resident springtails, and did not provide an appropriate model for field-weathered, Pb-contaminated soils. This indicates that physiochemical factors other than leaching may be more important determinants of Pb bioavailability. A 4-week exposure to Pb-amended soils containing up to 3,200 mg Pb/kg (nominal concentration) had no significant effect on *Sinella curviseta* springtail survival or reproduction (Xu et al., 2009a).

Carabid beetles (*Pterostichus oblongopunctatus*) inhabiting soils contaminated by pollution from a Pb-Zn smelter (containing 136 to 2,635 mg Pb/kg) were field-collected and then laboratory-reared for two generations (Lagisz and Laskowski, 2008). While fecundity was positively correlated to soil metal concentration (e.g., more eggs were produced by females collected from contaminated areas), the hatching rate of eggs diminished with increasing soil metal contamination. For the F1 generation, females produced by parents inhabiting highly polluted areas exhibited decreased body mass. The authors stated that these results indicate that invertebrates inhabiting metal- (or Pb-) contaminated soils could face "significantly altered life-history parameters." Similarly, aphids (*Brevicoryne brassicae*) reared on cabbage and radish plants exposed to 0.068 mg Pb daily exhibited altered development and reproduction when compared to those reared on non-exposed plants. Development time was increased by approximately two days, which led to a reduction in relative fecundity (Görür, 2007). Although the authors noted that study exposures were greater than what would be expected in naturally polluted areas, Pb exposure under field conditions could alter invertebrate life history patterns.

Several studies suggest that Pb may disrupt hormonal homeostasis in invertebrates. Shu et al. (2009) reported that vitellogenin production in both male and female *S. litura* moths was disrupted following chronic dietary exposure to Pb. Adult females reared on diets containing 25, 50, 100, or 200 mg Pb/kg exhibited decreased vitellogenin mRNA induction, and vitellogenin levels decreased with increasing Pb exposure. In addition, vitellogenin mRNA induction was detected in males exposed to 12 and 25 mg Pb/kg, and low levels of vitellogenin were found at those lower Pb exposures, when males normally do not produce any. In the Asian earthworm (*Pheretima guillelmi*), sperm morphology was found to be altered significantly following 2-week exposure to soils containing nominal concentration of 1,000, 1,400, 1,800, and 2,500 mg Pb/kg (Zheng and Li, 2009). Common deformities were swollen head and head helices, while head bending was also

recorded in some cases. These deformities were observed following exposures to concentration below the 14-day LC_{50} (3,207 mg Pb/kg) and below the concentration at which weight was diminished (2,800 mg Pb/kg). Experimentation with the model organism *Drosophila* indicates that Pb exposure may increase time to pupation and decrease pre-adult development, both of which are endocrine-regulated (Hirsch et al., 2010).

6.3.4.3 Terrestrial Vertebrates

Pb poisoning is one of the earliest recognized toxicoses of terrestrial vertebrates, occurring primarily through the ingestion of spent shot by birds. While the focus of the ISA is on more environmentally relevant exposures, studies of Pb poisoning provide historical context for the review. The widespread nature of this toxicosis was first noticed in American waterfowl around the turn of the last century [see Jones, (1939) for an historical summary]. Wetmore (1919) demonstrated that Pb shot caused the observed effects and described in detail the species affected, associated symptoms, and additional factors involved. By 1959, the estimated annual loss of waterfowl to Pb poisoning was 2−3 percent of the fall population (Bellrose, 1959). Smaller numbers of shorebirds and upland game birds were also found poisoned by Pb (Locke and Thomas, 1996).

The first reported Pb poisoning of a bald eagle (*Haliaeetus leucocephalus*) was described by Mulhern et al. (1970), and subsequently several hundred bald eagle Pb poisonings were diagnosed throughout the U.S. prior to the ban on use of Pb shot for waterfowl hunting (Kramer and Redig, 1997). Eagles and other raptors are poisoned by consuming Pb pellets imbedded in the flesh of ducks or upland prey species and may also be exposed to other sources of Pb, such as fishing sinkers and weights (Kramer and Redig, 1997). The use of Pb shot for waterfowl hunting was banned in 1991 due to the poisoning of bald eagles, which had been previously added to the endangered species list and were specially protected under the Bald Eagle Protection Act of 1940.

Anderson et al. (2000) reported that by 1997, mallard (*Anas platyrhynchus*) deaths from Pb poisoning in the Mississippi flyway were reduced by 64 percent, and ingestion of toxic pellets had declined by 78 percent. They estimated the ban prevented approximately 1.4 million duck deaths in the first 6-year period. However, Pb exposure remains widespread in bald eagles, although blood Pb concentrations have significantly decreased (Kramer and Redig, 1997). The endangered California condor (*Gymnogyps californianus*) also continues to have significantly elevated blood Pb levels as well as Pb-associated mortality resulting from exposure to ammunition fragments contained in food items (Cade, 2007; Church et al., 2006). Although there is a significant amount of

information on Pb tissue residues of mammals, there are very few reports of Pb poisoning; exceptions are reports of Pb poisoned bats in a cave in the southern U.S. and small mammals in the vicinity of several smelters (Shore and Rattner, 2001).

At the time of the 1977 Pb AQCD few studies of the effects of exposure to Pb had been conducted in wild animals other than birds, and the majority of those studies were of direct poisoning (U.S. EPA, 1977). Several studies of domestic animals grazing near Pb smelters indicated that horses are more susceptible than cattle to chronic Pb exposure although the findings were not conclusive due to the presence of other metals. Delta-aminolevulinic acid dehydratase (ALAD) was recognized as a sensitive indicator of Pb exposure in rats and waterfowl. In the 1986 Pb AQCD, additional effects of Pb on small mammals and birds were reported. According to the 2006 Pb AQCD (U.S. EPA, 2006b), commonly observed effects of Pb on avian and mammalian wildlife include decreased survival, reproduction, and growth, as well as effects on development and behavior. More recent experimental data presented here expand and support these conclusions, and also indicate that Pb can exert other effects on exposed terrestrial vertebrates, including alteration of hormones and other biochemical variables.

Since the 2006 Pb AQCD, there is additional evidence for hematological effects of Pb exposure in terrestrial vertebrates. Red-backed salamanders (*Plethodon cinereus*) exposed to Pb-amended soils (553, 1,700, 4,700, and 9,167 mg Pb/kg) by Bazar et al. (2010) exhibited lowered appetite and decreased white blood cell counts at the two highest concentrations, but tolerated field-collected, aged soils containing Pb concentrations of up to 16,967 mg Pb/kg with no significant deleterious effects. The white blood cell count of adult South American toads, (*Bufo arenarum)* was also decreased by weekly sublethal i.p. injections of Pb acetate at 50 mg Pb/kg body weight, (Chiesa et al., 2006). The toads also showed altered serum profiles and increased number of circulating blast cells. Final toad blood Pb levels were determined to be 8.6 mg Pb/dL, although it is unclear whether this is representative of Pb concentrations observed in field *B. arenarum* populations exposed to Pb. The authors suggested that, based on these findings, long-term environmental exposure to Pb could affect toad immune response. In western fence lizards (*S. occidentalis*), sub-chronic (60-day) dietary exposure to 10 to 20 mg Pb/kg per day resulted in significant sublethal effects, including decreased cricket consumption, decreased testis weight, decreased body fat, and abnormal posturing and coloration (Salice et al., 2009). Long-term dietary Pb exposures are thus likely to decrease lizard fitness.

Even in cases of high environmental Pb exposures, however, linking Pb body burdens to biological effects can be difficult. Pb concentration in the breast feathers, washed tail feathers, and blood of field-collected blackbirds (*Turdus merula*) were determined to be

3.2 mg Pb/kg, 4.9 mg Pb/kg, and 0.2 mg Pb/kg wet mass in urban birds, as opposed to 1.4 mg Pb/kg, 1 mg Pb/kg, and 0.05 mg Pb/kg in rural birds (Scheifler et al., 2006a). However, the elevated Pb tissue concentrations in urban birds were not significantly correlated to any index of body condition.

The long-term effect of atmospheric Pb deposition on pied flycatcher (*Ficedula hypoleuca*) nestlings was determined in native communities residing in the Laisvall mining region of Sweden (Berglund et al., 2010). Moss samples indicated that Pb deposition in study areas ranged between 100 and 2,000 mg Pb/kg dry weight during operations and 200 and 750 mg Pb/kg when operations ceased. A simultaneous slight reduction was observed in pied flycatcher blood Pb levels, from 0.4 to 0.3 mg Pb/kg. However, clutch size was decreased in pied flycatchers inhabiting the mining area both during and after mining operations, and mean nestling mortality was 2.5 times higher in the mining region than in reference areas during mining operations, and 1.7 higher five years after cessation of mining operations. The authors noted that Pb deposition in the mining region remained elevated even after mining operations ceased, and that stable Pb isotope analysis suggested that smelter Pb remained available to pied flycatcher through the transfer of historically deposited Pb in soil to prey items.

Berglund et al. (2010) also analyzed ALAD activity in pied flycatchers at the later period, and found that it was 46% lower at the mine site. Beyer et al. (2004) observed that elevated blood Pb levels in several types of birds inhabiting the Tri-State Mining District (Oklahoma, Kansas, Missouri) were correlated with decreases in ALAD activity. Based on reduction in ALAD activity, robins (*Turdus migratorius*) were most sensitive to Pb exposure (35% reduction), followed by cardinals (*Cardinalis cardinalis*), waterfowl, and bobwhite quail (*Colinus virginianus*) (40%, 41%, and 56% reductions, respectively). Eagle owl (*Bubo bubo*) nestlings living in a historical mining area in Spain also exhibited elevated blood Pb levels (average 8.61 µg/dL as compared to an average reference area value of 3.18 µg/dL), and this was correlated to an approximate 60% reduction in ALAD activity (Gómez-Ramírez et al., 2011). Hansen et al. (2011a) determined that ground-feeding songbirds were frequently exposed to Pb within the Coeur d'Alene, ID mining region. Robins, in particular, were significantly likely to exhibit blood Pb levels in the clinical and severe clinical poisoning ranges (50 to 100 µg/dL and >100 µg/dL, respectively). Ingested soil Pb accounted for almost all of the songbirds' exposure to Pb, with Pb exposure correlated with estimated soil ingestion rates (20% for robins, 17% for song sparrows, and 0.7% for Swainson's thrushes, *Catharus ustulatus*). More than half of the robins and song sparrows from all contaminated sites and more than half of the Swainson's thrushes from highly contaminated sites showed at least 50% inhibition of ALAD. The highest hepatic Pb concentration of 61 mg/kg (dry weight) was detected in a song sparrow (Hansen et al., 2011a).

Blood Pb was significantly elevated in waterfowl in the Lake Coeur d'Alene areas of Blackwell Island and Harrison Slough (mean sediment concentrations of 679 and 3,507 mg Pb/kg dry weight, respectively). Twenty-seven percent of the waterfowl sampled in the Blackwell Island region had blood Pb concentrations suggestive of severe clinical poisoning (average concentration=0.17 mg Pb/kg); in the Harrison Slough, 60% of sampled waterfowl had highly elevated blood Pb levels that exceeded the severe clinical poisoning threshold (average concentration=2.2 mg Pb/kg) (Spears et al., 2007). The level of corticosteroid hormones in field populations of white stork nestlings (*Ciconia ciconia*) in a mining area affected by Pb and other metals was positively correlated with blood Pb levels (Baos et al., 2006). The effect was more pronounced for single nestlings than for multiple-chick broods. Surprisingly, average blood Pb levels in chicks inhabiting reference areas was 910 µg Pb/dL (± 51), which was higher than blood Pb levels from the mining area (440 ± 340 µg Pb/dL). However, the correlation between blood Pb levels and the corticosteroid stress response in white stork nestlings was observed in both groups of birds. Burger and Gochfeld (2005) exposed herring gull (*Larus argentatus*) chicks to Pb acetate via an i.p. injection of 100 mg Pb/kg body weight, to produce feather Pb concentration approximately equivalent to those observed in wild gulls. Pb-exposed gulls exhibited abnormal behaviors, including decreased walking and food begging, erratic behavioral thermoregulation, and diminished recognition of caretakers. Interestingly, subchronic exposure of Japanese quail (*Coturnix coturnix japonica*) to 5 and 50 mg Pb/L in drinking water caused an increase in their immune response. Exposed quail exhibited significantly lower rates of death or health effects (including septicemia, perihepatitis, and pericarditis among others) than control animals following infection with *Escherichia coli*, and the incidence of infection-related effects was dependent on Pb exposure (Nain and Smits, 2011). These observations contrast with immunotoxicology results in mice reported in Section 4.6.5.1.

Again, dietary or other health deficiencies unrelated to Pb exposure are likely to exacerbate the effects of Pb. Ca^{2+}-deficient female zebra finches (*T. guttata*) had a suppressed secondary humoral immune response following 28-day exposures to 20 mg Pb/L in drinking water (Snoeijs et al., 2005). This response, however, was not observed in birds fed sufficient Ca^{2+}. Although a significant finding, these data are difficult to interpret under field conditions where the overall health of avian wildlife may not be easily determined.

Chronic Pb exposures were also demonstrated to affect several mammalian species. Young adult rats reared on a diet containing 1,500 mg Pb/kg Pb acetate for 50 days demonstrated less plasticity in learning than non-exposed rats (McGlothan et al., 2008), indicating that Pb exposure caused significant alteration in neurological function. Yu et al. (2005) showed that dietary Pb exposure affected both the growth and endocrine

function of gilts (*S. domestica*). Consumption of 10 mg Pb/kg diet resulted in lower body weight and food intake after 120 days of dietary exposure; Pb exposure decreased final weight by 8.2%, and average daily food intake of Pb-exposed pigs was decreased by 6.8% compared to control intake. Additionally, concentration of estradiol, luteinizing hormone, and pituitary growth hormone were decreased (by 12%, 14%, and 27% versus controls, respectively), while blood Pb level was increased by 44% to an average 2.1 µg/dL. In cattle grazing near Pb-Zn smelters in India, blood Pb levels were positively correlated with plasma levels of the thyroid hormones thyroxine (T4) and tri-iodothyronine (T3) and the hepatic biomarkers alanine transaminase and aspartate transaminase (Swarup et al., 2007). Total lipids, total protein and albumin levels were decreased in the same animals. Rodriguez-Estival et al. (2011) determined that red deer (*Cervus elaphus*) and wild boar (*Sus scrofa*) inhabiting a Pb-contaminated mining area in Spain exhibited increased liver and bone Pb concentrations (geometric means of 0.35 and 0.46 mg Pb/kg for red deer, and 0.81 and 7.36 mg Pb/kg for wild boar, respectively). These tissue concentrations were correlated to a significant decrease in red deer glutathione production, but corresponded to an increase in wild boar glutathione (Rodríguez-Estival et al., 2011). Authors proposed that the different antioxidant responses may be indicative of different Pb susceptibilities in the two species.

Following previous reports of in vivo follicle and oocyte damage in animals with low-level Pb accumulation, Nandi et al. (2010) treated oocytes of buffalo (*Bubalus bubalis*) in vitro with Pb at concentrations ranging from 0.005 to 10 mg/L in one-day cultures indicated a significant decline in viability of oocytes at 1 mg/L. Dose-dependent effects on oocyte viability, morphological abnormalities, cleavage, blastocyst yield and blastocyst hatching were observed in Pb-treated oocytes with maturation significantly reduced at 2.5 mg/L and 100% oocyte death at 32 mg/L. These results appear to confirm previous reports, but the in vitro concentrations of Pb are difficult to relate to in vivo exposures. On the other hand, the reproductive viability of wild red deer from the Pb-contaminated mining area of Spain studied by Rodriguez-Estival et al. (2011) was shown to be altered, with 11% and 15% reductions in spermatozoa and acrosome integrity observed in male deer from the mining area compared with those residing in reference areas (Reglero et al., 2009a).

6.3.5 Exposure and Response of Terrestrial Species

Evidence regarding exposure-response relationships and potential thresholds for Pb effects on terrestrial populations can inform determination of standard levels that are protective of terrestrial ecosystems. Given that exposure to Pb may affect plants, invertebrates, and vertebrates at the organism, population, or community level,

determining the rate and concentration at which these effects occur is essential in predicting the overall risk to terrestrial organisms. This section updates available information derived since the 2006 Pb AQCD, summarizing several dose-response studies with soil invertebrates. As shown in the studies summarized in Table 6-4 and Figure 6-4, several experiments have been published that used multiple levels of Pb under controlled conditions. However, none of them treated Pb concentration as a continuous variable, i.e., none attempted to analyze results as a concentration–response relationship. In addition, given the well-established presence of strong interactions with variables such as pH, CEC, OC, or aging, applying exposure–response relationships from those experiments to natural conditions with different values of those interacting variables could be difficult.

Dose-dependent responses in antioxidant enzymes were observed in adult *L. mauritii* earthworms exposed to soil-associated Pb contamination (75, 150, 300 mg Pb/kg) (Maity et al., 2008). By day seven of exposure, glutathione-S-transferase activity and glutathione disulfide concentration were positively correlated with increasing Pb exposures, while glutathione concentration exhibited a negative dose-response relationship with soil Pb concentration. However, these trends had become insignificant by the end of the total exposure period (28 days), as a result of normalization of antioxidant systems following chronic exposure. This strongly suggests that changes to earthworm antioxidant activity are an adaptive response to Pb exposures.

Both survival and reproductive success of *E. fetida* earthworms showed concentration-dependent relationships with soil Pb concentration during the course of standard 14- and 56-day toxicity tests (Jones et al., 2009b). Five levels of Pb soil concentration were prepared for the acute 14-day study via spiking with Pb nitrate—0, 300, 711, 1,687, and 2,249 mg Pb/kg, while soil concentration of 0, 355, 593, 989, and 1,650 mg Pb/kg were used in chronic (56-day) earthworm bioassays. A 14-day acute LC_{50} of 2,490 mg Pb/kg was determined from the dose-response relationship, while the approximate 56-day NOEC (no observed effect concentration) and EC_{50} values were about 400 mg Pb/kg and 1,000 mg Pb/kg, respectively. Jones et al. (2009b) made use of continuous (regressional) models to characterize the relationship between Pb soil concentration and Pb accumulation in earthworms, but did not use continuous models for the relationship of exposure and other responses. Currie et al. (2005) observed mortality of *E. fetida* after 7 and 14 days in spiked field soil at seven levels of Pb (0 to 10,000 mg Pb/kg). They reported LC_{50} values of 2,662 mg Pb/kg at 7 days and 2,589 mg Pb/kg at 14 days or 2,827 mg Pb/kg at both 7 and 14 days, depending on the number of worms in the experimental enclosure.

Other studies have shown no correlation between Pb concentration in either earthworm tissue or soil, and earthworm survival rate. Although the Pb content of *E. fetida* held in metal-contaminated soils containing between 9.7 and 8,600 mg Pb/kg was positively correlated with Pb concentration of fully aged soil collected from disused mines, there was no statistical relationship with earthworm survival during a 42-day exposure period (Nahmani et al., 2007). However, Pb concentration in soil leachate solution was significantly correlated with decreased earthworm survival and growth (linear regression: R^2= 0.64, p<0.0001). The 42-day Pb EC_{50} for *E. fetida* growth was 6,670 mg Pb/kg.

Langdon et al. (2005) exposed three earthworm species (*E. andrei*, *L. rubellus*, and *A. caliginosa*) to Pb nitrate-amended soils at concentrations of 1,000 to 10,000 mg Pb/kg to determine species variability in uptake and sensitivity. Twenty-eight-day LC_{50} values for the three species were 5,824 mg Pb/kg, 2,867 mg Pb/kg, and 2,747 mg Pb/kg, respectively, indicating that *L. rubellus* and *A. caliginosa* are significantly more vulnerable to Pb contamination than *E. andrei*, a common laboratory species. This is comparable to previous findings by Spurgeon et al. (1994) who reported 14-day LC_{50} of 4,480 mg Pb/kg and 50-day LC_{50} of 3,760 mg Pb/kg for *E. fetida*, another standard laboratory test species. In the more recent study of *E. fetida* sensitivity summarized above, Jones et al. (2009b) reported LC_{50} values for *E. fetida* that are similar to those for *L. rubellus* and *A. caliginosa*. It is likely that these apparent species differences are a result of differential bioavailability of the Pb in test soils. However, the Pb body burden of all three species in the study by Langdon et al. (2005) increased with increasing environmental concentration, and there were no species differences in Pb tissue content. When given a choice between treated and untreated soils, all worm species exhibited significant avoidance of Pb-contaminated soils, and altering pH (and, consequently, Pb bioavailability) had no impact on avoidance (Langdon et al., 2005). Field earthworms may thus be able to reduce their exposure to Pb through behavior.

Reproductive success of other soil invertebrates is impacted by Pb. The organismal and population-level responses of the springtail *Paronychiurus kimi* to Pb were determined by Son et al. (2007) using artificial soils, following the 1999 ISO methodology. The 7-day Pb LC_{50} was determined to be 1,322 mg Pb/kg dry weight, while the 28-day reproduction EC_{50} was established as 428 mg Pb/kg. The intrinsic rate of population increase was lower at a Pb soil concentration of 1,312 mg Pb/kg, and the authors estimated that, at this level, *P. kimi* populations would be extirpated. The authors noted that, in this case, the reproductive endpoint overestimated the population-level risk for *P. kimi* springtails exposed to Pb, and proposed that more specific measures of population-level endpoints (such as the reduction in intrinsic rate of increase) be used to determine risk to populations. Menta et al. (2006) showed that a nominal soil concentration of 1,000 mg Pb/kg decreased the reproductive output of two collembolans, *Sinella coeca* and

F. candida. Pb concentrations of 50, 100, and 500 mg Pb/kg slightly but significantly depressed *S. coeca* adult survival, while *F. candida* survival was statistically unaffected by Pb exposure. The hatching success of *F. candida* eggs was diminished by 10-day exposure to Pb-spiked soils; the 10-day EC_{50} for hatching success was reported as 2,361 mg/kg Pb (Xu et al., 2009b). However, authors noted that egg development was more sensitive to Cu and Zn exposure, and by comparison, was less susceptible to Pb.

In addition to species variability, physical and chemical factors affecting Pb bioavailability were also demonstrated to significantly influence the toxicity of Pb to terrestrial species. As noted previously in Section 6.3.2, laboratory-amended artificial soils provide a poor model for predicting the toxicity of Pb-contaminated field soils, because aging and leaching processes, along with variations in physiochemical properties (pH, CEC, OM), influence metal bioavailability. Consequently, toxicity values derived from exposure-response experimentation with laboratory-spiked soils probably overestimate true environmental risk, with the possible exception of highly acidic sandy soils. Because toxicity is influenced by bioavailability and soil biogeological and chemical characteristics, extrapolation of toxic concentrations between different field-collected soils will be difficult. Models that account for those modifiers of bioavailability, such as the terrestrial BLM proposed by Smolders et al. (2009), have proven difficult to develop due to active physiological properties of soil organisms affecting either uptake (such as root phytochelatins) or sequestration of Pb (such as granule formation in root tissues and earthworms, or substitution of Pb for calcium in bones).

6.3.6 Terrestrial Community and Ecosystem Effects

A study reviewed in the 1977 Pb AQCD provided evidence for Pb effects on forest-nutrient cycling and shifts in community composition. Reduced arthropod density, biomass and richness were observed in the vicinity of a smelting complex in southeastern Missouri where Pb, Cd, Zn and Cu were measured in the litter layer and soil (U.S. EPA, 1977; Watson et al., 1976). In the 1986 Pb AQCD it was reported that Pb at environmental concentrations occasionally found near roadsides and smelters (10,000 to 40,000 mg Pb/kg dry weight) can eliminate populations of bacteria and fungi on leaf surfaces and in soil. At soil concentrations of 500 to 1,000 mg Pb/kg or higher, populations of plants, microorganisms, and invertebrates may shift toward Pb-tolerant populations of the same or different species (U.S. EPA, 1986b).

According to the 2006 Pb AQCD (U.S. EPA, 2006b), natural terrestrial ecosystems near significant Pb stationary sources (such as smelters and mines) exhibited a number of ecosystem-level effects, including decreased species diversity, changes in floral and

faunal community composition, and decreasing vigor of terrestrial vegetation. These findings are summarized in Table AX7-2.5.2 of the Annex to the 2006 Pb AQCD (U.S. EPA, 2006c). More recent literature explored the interconnected effects of Pb contamination on soil bacterial and fungal community structure, earthworms, and plant growth, in addition to impacts on soil microbial community function.

Inoculation of maize plants with *Glomus intraradices* arbuscular mycorrhizal fungi isolates decreased Pb uptake from soil, resulting in lower shoot Pb concentration and increased plant growth and biomass (Sudova and Vosatka, 2007). Similarly, Wong et al. (2007) showed that the presence of arbuscular mycorrhizal fungi improved vetiver grass (*Vetiveria zizanioides*) growth, and while Pb uptake was stimulated at low soil concentration (10 mg Pb/kg), it was depressed at higher concentration (100 and 1,000 mg Pb/kg). Bojarczuk and Kieliszewska-Rokicka (2010) found that the abundance of ectomycorrhizal fungi was negatively correlated with the concentration of metals, including Pb, in the leaves of silver birch seedlings. Arbuscular mycorrhizal fungi may thus protect plants growing in Pb-contaminated soils. Microbes too may dampen Pb uptake and ameliorate its deleterious effects: biomass of plants grown in metal-contaminated soils (average Pb concentration 24,175 mg Pb/kg dry weight) increased with increasing soil microbial biomass and enzymatic activity (Epelde et al., 2010). However, above certain Pb concentration, toxic effects on both plants and microbial communities may prevent these ameliorating effects. Yang et al. (2008b) found that both the mycorrhizal colonization and the growth of *Solidago canadensis* were negatively affected by soil Pb contamination. They suggested that, more generally, Pb-mediated alterations in plant-fungal dynamics may be the cause of ecological instability in terrestrial vegetative communities exposed to metals.

The presence of both earthworms and arbuscular mycorrhizal fungi decreased the mobility of Pb in mining soils undergoing phytoremediation (Ma et al., 2006). Inoculation with both earthworms and fungi increased plant growth at sites contaminated with mine tailings compared to that observed at sites with 75% less Pb contamination. Most likely, this was a result of the decrease in bioavailable (DTPA-extractable and ammonium acetate-extractable) Pb to 17% to 25% of levels in areas without the earthworm and arbuscular mycorrhizal fungi amendments. The presence of earthworms in metal-contaminated soils decreased the amount of water-soluble Pb (Sizmur and Hodson, 2008), but despite this decrease, ryegrass accumulated more Pb from earthworm-worked soils than soils without worms present. Sizmur and Hodson speculated that increased root dry biomass may explain the increased uptake of Pb in the presence of earthworms. However, Sizmur et al. (2011) found that the presence of anecic (deep-burrowing) earthworms (*L. terrestris*) increased soil leachate Pb concentrations by 190%. The authors observed that worms promoted a faster breakdown of organic matter,

which caused a decrease in soil pH and a concurrent increase in Pb solubility. As a result, ryegrass (*L. perenne*) accumulated a greater amount of Pb in systems with earthworms (Sizmur et al., 2011). Further, the presence of earthworms (*Lumbricus terrestris*) was found to increase Pb concentrations in both maize and barley, although growth of these species was unaffected (Ruiz et al., 2011). Authors noted that worm activity increased Pb extraction yields by factors of 4.4 and 7.6, for barley and maize. By contrast, Coeurdassier et al. (2007) found that Pb was higher in earthworm tissue when snails were present, but that snails did not have a higher Pb content when earthworms were present.

Microbial communities of industrial soils containing Pb concentrations of 61, 456, 849, 1,086, and 1,267 mg Pb/kg dry weight were also improved via revegetation with native plants, as indicated by increased abundances of fungi, actinomycetes, gram-negative bacteria, and protozoa, as well as by enhanced fatty acid concentration (Zhang et al., 2006). Increased plant diversity ameliorated the effects of soil Pb contamination (300 and 600 mg Pb/kg) on the soil microbial community (Yang et al., 2007).

The effect of Pb on microbial community function has been quantified previously using functional endpoints such as respiration rates, fatty acid production, and soil acid phosphatase and urease activities, which may provide an estimate of ecological impacts separate from microbial diversity and abundance measurements. Most studies of metal-induced changes in microbial communities have been conducted using mixtures of metals. However, Akerblom et al. (2007) tested the effects of six metals (Cr, Zn, Mo, Ni, Cd, and Pb) individually. All tested metals had a similar effect on the species composition of the microbial community. Exposure to a high Pb concentration (52 mg Pb/kg) also negatively affected respiration rates. Total phospholipid fatty acid content was determined to negatively correlate with increasing Pb exposure, indicating alteration of the microbial community. When Yang et al. (2006) compared the microbial properties of metal-contaminated urban soils to those of rural soils, significant differences were detected in basal community respiration rates and microbial abundance. The urban soils studied contained multiple metal contaminants, but microbial biomass was the only measured endpoint to be significantly and negatively correlated to Pb concentration. Similarly, the fungal community in a naturally Pb-enriched forest in Norway exhibited differences in community composition and abundance when compared with other, low Pb sites. The number of colony–forming fungal units was diminished by soil Pb, and was approximately 10 times lower in the highest Pb soil concentration (~4300 mg Pb/kg). Further, only one fungus species was isolated from both high Pb and control soils, indicating highly divergent communities; species diversity was also reduced by high soil Pb concentrations (Baath et al., 2005). These studies suggest that anthropogenic Pb contamination may affect soil microbial communities, and alter their ecological function. However, (Khan et al., 2010c) reported that it is possible for indicators of microbial

activity to recover after an initial period depression. (Khan et al., 2010c) found that following a 2-week exposure to three levels of Pb (150, 300, and 500 mg Pb/kg), the number of culturable bacteria at the highest exposure concentration tested was decreased. Acid phosphatase and urease levels (measures of soil microbial activity) decreased significantly, but they had recovered by the ninth week. Another study (Bamborough and Cummings, 2009) reported that no changes in bacterial and actinobacterial diversity in metallophytic soils containing 909 to 5,280 mg Pb/kg (43 to 147 mg Pb/kg bioavailable Pb (as defined by the study authors)). Soil bacteria community structure and basal respiration rates were examined in natural soils with pH values ranging from 3.7 to 6.8 (Lazzaro et al., 2006). Six soil types of differing pH were treated with Pb nitrate concentrations of 0.5, 2, 8, and 32 mM (104, 414, 1,658, and 6,630 mg Pb/L). Basal respiration was decreased in two soil types tested at the highest Pb treatment (32 mM, =6,630 mg Pb/L), and in a third at the two highest Pb treatments (8 and 32 mM, =1658 and 6,630 mg Pb/L). Terminal Restriction Fragment Length Polymorphism analysis indicated that bacterial community structure was only slightly altered by Pb treatments. While pH was correlated with the amount of water-soluble Pb, these increases were apparently not significant enough to affect bacterial communities, because there were no consistent relationships between soil pH and respiration rate or microbial community structure at equivalent soil Pb concentration. Pb contamination was also demonstrated to reduce phenol oxidase activity in several types of soils; when treated with Pb as Pb acetate in concentrations between 5 and 50 nM Pb (0.001 and 0.01 mg Pb/L), phenol oxidase activity significantly decreased in all soils tested, while 400 nM (0.08 mg Pb/L) and greater completely arrested phenol oxidase activity in one soil tested (a high pH sandy loam) (Carine et al., 2009). Carine et al. (2009) suggested that the decreased soil enzymatic activity resulted from changes in the microbial community following Pb exposure. Pb concentrations between 50 and 500 mg Pb/kg significantly reduced microbial abundance and diversity, and also resulted in lower soil phosphatase, urease, and dehydrogenase activities (Gao et al., 2010b). Further, the weekly soil carbon dioxide evolution rate was significantly reduced by concentrations of 5, 10, and 50 mg Pb/kg, which also indicated decreased microbial respiration and adverse effects on the microbial community (Nwachukwu and Pulford, 2011). Gai et al. (2011) examined the microbial activity of three soils via microcalorimetric methods following Pb exposure. They noted an increase in activity immediately following Pb application (giving 10, 20, 40, 80, and 160 mg Pb/kg), and theorized that this was a result of rapid mortality of sensitive microbial species, followed by a concurrent proliferation of Pb-tolerant microorganisms. As Pb concentrations increased, however, the calculated microbial growth rate constant decreased, indicating a suppression of microbial activity (Gai et al., 2011). Authors also noted a strong correlation between microcalorimetry estimates and the number of colony forming units isolated from soil samples.

Pb exposure negatively affected the prey capture ability of certain fungal species. Nematophagous fungi are important predators of soil-dwelling nematodes, collecting their prey with sticky nets, branches, and rings. The densities of traps they constructed decreased in soils treated with 0.15 mM Pb chloride (31 mg Pb/L) (Mo et al., 2008). This suppression caused a subsequent reduction in fungal nematode capturing capacity, and could result in increased nematode abundance.

In a study of microbial communities and enzyme activity, Vaisvalavicius et al. (2006) observed that high concentration of soil metals were linked to a significant reduction in soil microorganism abundance and diversity. Soil columns spiked with Cu, Zn, and Pb acetate (total Pb concentration of 278 to 838 mg Pb/kg, depending on depth) exhibited a 10- to 100-fold decrease in microbial abundance, with specific microbe classes (e.g., actinomycetes) seemingly more affected than others (Vaisvalavicius et al., 2006). Concurrently, decreases in soil enzymatic activity were also observed, with saccharase activity decreased by 57–77%, dehydrogenase activity by 95–98%, and urease activity 65–97%. Although this suggests that Pb contamination may alter the nutrient cycling capacity of affected soil communities, it is difficult to separate the impact of Pb in this study from the contributions of Cu and Zn that were also added. In contrast, Zeng et al. (2007) reported that soil concentrations of 300 mg Pb/kg and less stimulated soil enzymatic activity. Both urease and dehydrogenase levels were increased and rice dry weight was unaffected by concentrations of 100 and 300 mg Pb/kg. However, at 500 mg Pb/kg, both rice and soil enzyme activities and microbial biomass were decreased suggesting impacts at the community level for the soil-rice system. The authors proposed that these concentrations could be considered the critical Pb concentration in rice paddy systems (Zeng et al., 2007).

The microbial communities of soils collected from a Pb-Zn mine and a Pb-Zn smelter were significantly affected by Pb and other metals (e.g., Cd) (Hu et al., 2007b). At a mine site, Pb concentration of 57 to 204 mg Pb/kg and Cd concentration of 2.4 to 227 mg Cd/kg decreased the number of bacteria-forming colonies extracted from soils. Principal component analysis of microbial community structure demonstrated that different communities were associated with different metal soil concentration. Similarly, soil microbial communities exposed to metal contamination from a smelter site (soil Pb concentration ranging from 30 to 25,583 mg Pb/kg dry weight) showed decreased bacterial functional diversity (although fungal functional diversity increased) and no effects on soil respiration rates were observed (Stefanowicz et al., 2008). This led the authors to conclude that bacterial diversity is a more sensitive endpoint and a better indicator of metal exposure than fungal diversity or microorganism activity. In a similar study, Kools et al. (2009) showed that soil ecosystem variables measured after a 6-month

exposure to metal-contaminated soil indicated that Pb concentration (536 or 745 mg Pb/kg) was an important driver of soil microbial species biomass and diversity.

Pb-resistant bacterial and fungal communities were extracted regularly from soil samples at a shooting range site in southern Finland (Hui et al., 2009). While bioavailable Pb concentration averaged 100 to 200 mg Pb/kg as determined by water extraction, the total Pb concentrations measured on site were 30,000 to 40,000 mg Pb/kg. To determine Pb tolerance, bacterial colonies extracted and cultured from shooting range and control soils were grown on media containing either 0.4 or 1.8 mM Pb (83 or 373 mg Pb/L). While bacteria isolated from control soil did not proliferate on high-Pb media, shooting-range soil microbe isolates grew on high-Pb media and were deemed Pb tolerant. The authors noted that bacterial species common in control samples were not detected among the Pb-tolerant species isolated from shooting-range soils. They speculated that if long-term exposure to minimally bioavailable Pb can alter the structure of soil decomposer communities, decomposition rates could be altered. However, this would require that the microbial ecosystem decomposing function be altered along with structure, and the authors provided no evidence for alteration of function.

Microbial communities associated with habitats other than soils are also affected by exposure to atmospherically deposited Pb. Alder (*Alnus nepalensis*) leaf microorganism populations were greater in number at non-affected sites than at sites adjacent to a major Indian highway with increased Pb pollution (Joshi, 2008). The density, species richness, and biomass of testate amoebae communities grown on *Sphagnum fallax* mosses were significantly decreased following moss incubation in Pb solutions of either 0.6 or 2.5 mg Pb/L (Nguyen-Viet et al., 2008). More importantly, species richness and density were negatively correlated with Pb concentration accumulated within the moss tissue. The structure of microbial communities associated with lichen surfaces was affected by lichen trace-element accumulation, including Pb content. Lichens collected from industrial areas had elevated Pb concentration (10 to 20 mg Pb/kg versus 5 to 7 mg Pb/kg in urban and rural areas, respectively) and housed bacterial communities characterized by increased cyanobacteria biomass (Meyer et al., 2010).

Following a 28-day exposure to field-collected soils contaminated with metals (including Pb at 426 mg Pb/kg), both population growth and individual growth of the earthworm *L. rubellus* were diminished (Klok et al., 2006). The authors proposed that, although these reductions were unlikely to result in extirpation, avian predators such as the godwit (*Limosa limosa*) that feed heavily on earthworms may be affected by a reduction of available earthworm biomass.

During the past 5 years, there has been increasing interest in the effects of Pb and other metals on the functional aspects of soil microbial communities. Most studies show that

Pb decreases diversity and function of soil microorganisms. However, in an example of ecological mutualism, plant-associated arbuscular mycorrhizal fungi were found to protect the host plant from Pb uptake, while fungal viability is protected by the host plants. Similarly, soil microbial communities (bacterial species as well as fungi) in Pb-contaminated soils are improved by revegetation. A few studies have reported on effects of Pb to populations of soil invertebrates. They demonstrated that Pb can decrease earthworm population density, although not to levels that would result in local extinction. There have been no recently reported studies on the potential effects of Pb on terrestrial vertebrate populations or communities, or possible indirect effects through reduction of prey items such as earthworms.

6.3.7 Critical Loads in Terrestrial Systems

The general concept and definition of critical loads is introduced in Section 6.1.3 of this chapter [also see Section 7.3 of the 2006 Pb AQCD (U.S. EPA, 2006c)]. An international workshop was conducted in 2005 on the development of critical loads for metals and other trace elements (Lofts et al., 2007). Among the findings of the workshop it was reported that soil transport and transformation processes are key in controlling the fate of metals and trace elements, thus their importance in the input-output mass balance needs to be considered. The degree to which these processes are understood and can be quantified varies. Complexation, sorption, ion exchange and precipitation are well understood under laboratory conditions, but to a lesser extent in the field (Lofts et al., 2007). Slower processes of weathering and fixation are less well understood or studied than leaching (Lofts et al., 2007).

As noted in previous sections, soil pH and organic matter influence Pb availability. De Vries et al. (2007) demonstrate that critical limits, measured as critical reactive metal content, can significantly vary between soil types that differ in pH and organic matter. Critical limits of Pb increased from 30 to 64 (mg Pb/kg) over a pH range of 4-7 when soil organic matter content was 5%, while these limits increased from 187 to 400 (mg Pb/kg) over the same pH range when organic content was 80%. These implications suggest that critical limits increase with increasing soil organic matter. This has important consequences for forest soils because many are covered by an organic layer where roots, fungi and other microorganisms are located. Baath (1989) evaluated the effects of organic matter on critical limits for microorganisms, measured via enzyme synthesis, litter decomposition and soil respiration. Results indicate critical limits are up to four times higher in the organic (135 to 976 mg Pb/kg) than the mineral soil layer (32 to 690 mg Pb/kg) at hazardous concentration ranging from 5-50% of species. In general, De Vries

et al. (2007) found support that ecotoxicological critical limits in European soils for Pb decrease with increasing pH.

Several methods are routinely used for Pb risk assessment of terrestrial animals. Buekers et al. (2009) proposed the use of a Tissue Residue Approach as a risk estimation method for terrestrial vertebrates that eliminates the need for quantitative estimation of food intake or Pb species bioavailability. Blood Pb no observed effect concentration (NOEC) and lowest observed effects concentration (LOEC) data derived from 25 studies examining the effects of Pb exposure on growth, reproduction, and hematological endpoints were used to construct a series of species sensitivity distributions for mammals and birds. They also used the HC5 criterion (5th percentile of species NOEC values for collection of species) proposed by Aldenberg and Slob (1993). For mammals, the HC5 values obtained ranged from 11 to 18 µg Pb/dL blood; HC5 values for birds ranged from 65 to 71 µg Pb/dL. The authors proposed the use of 18 and 71 µg Pb/dL as critical threshold values for mammals and birds respectively, which are below the lowest NOEC for both data sets used, and are above typical background Pb values. It is difficult to determine environmental Pb toxicity given the variation of physicochemical and soil properties that alter bioavailability and toxicity. This variability makes it difficult to extrapolate between areas. Furman et al. (2006) proposed the use of a physiologically based extraction test to predict risks posed to waterfowl from environmental Pb contamination. The extraction process was modeled after gastric and intestinal conditions of waterfowl, and was used to gauge the bioavailability of Pb from freshly amended and aged contaminated soils. The concentration of Pb extracted through the use of the physiologically based extraction test was demonstrated to be significantly correlated to Pb tissue concentration in waterfowl exposed via in vivo studies of the same soils.

No critical loads for Pb from terrestrial ecosystems in the U.S. were identified for review in this ISA; however, work has been conducted in Europe. Given that local conditions (including historic loading, soil transport and transformation processes) are key elements to critical load calculation the utility of critical loads that are developed from other countries for application to U.S. ecosystems is unclear. The most recent European publications on Pb critical loads include assessments of the U.K., Netherlands and Italy. Hall et al. (2006) used the critical load approach to conduct a national risk assessment of atmospheric Pb deposition for the U.K. While specific regions were determined to have low critical load values for Pb (central England [Peak district area], the Pennines [in north-central England], in southern Wales, and scattered areas in other parts of England, Wales, and Scotland), the authors noted that this approach can be significantly biased, as available ecotoxicological data used in the modeling were from studies that were not conducted in soils representative of all U.K. soils. De Vries and Groenenberg (2009) similarly observed that the uncertainty inherent in a critical load approach to Pb risk

assessment is influenced by the critical concentration of dissolved metal and the absorption coefficients of exposed soils. However, this approach did indicate that for forest soils in the Netherlands, 29% of the areas would be expected to exceed the critical load, based on currently available toxicity data and Pb pollution data (de Vries and Groenenberg, 2009). Similarly, although Pb soil concentrations in the Bologna Province of Italy were far below concentrations harmful to soil organisms, current atmospheric Pb deposition rates suggest that critical load exceedances are likely in the future, unless annual Pb emissions are decreased (Morselli et al., 2006).

Given the heterogeneity of ecosystems exposed to Pb, and the differences in expectations for ecosystem services attached to different land uses, it is expected that critical load values for Pb for soils within the U.S. would fall within a broad range. Refer to Section 7.3.5 of the 2006 Pb AQCD for additional discussion of critical loads of Pb in terrestrial systems.

6.3.8 Soil Screening Levels

Developed by EPA, ecological soil screening levels (Eco-SSLs) are maximum contaminant concentrations in soils that are predicted to result in little or no quantifiable effect on terrestrial receptors. These conservative values were developed so that contaminants that could potentially present an unacceptable hazard to terrestrial ecological receptors are reviewed during the risk evaluation process while removing from consideration those that are highly unlikely to cause significant effects. The studies considered for the Eco-SSLs for Pb and detailed consideration of the criteria for developing the Eco-SSLs are provided in the 2006 Pb AQCD (U.S. EPA, 2006c). Preference is given to studies using the most bioavailable form of Pb, to derive conservative values. Soil concentration protective of avian and mammalian diets are calculated by first converting dietary concentration to dose (mg/kg body weight per day) for the critical study, then using food (and soil) ingestion rates and conservatively derived uptake factors to calculate soil concentration that would result in unacceptable dietary doses. This frequently results in Eco-SSL values below the average background soil concentration [19 mg Pb/kg dry weight (U.S. EPA, 2005b, 2003b)], as is the case with Pb for birds. The Pb Eco-SSL was completed in March 2005 and has not been updated since. Values for terrestrial birds, mammals, plants, and soil invertebrates are 11, 56, 120, and 1,700 mg Pb/kg soil (dry weight), respectively.

6.3.9 Characterization of Sensitivity and Vulnerability

Research has long demonstrated that Pb affects survival, reproduction, growth, metabolism, and development in a wide range of species. The varying severity of these effects depends in part upon species differences in metabolism, sequestration, and elimination rates. Dietary factors also influence species sensitivity to Pb. Because of effects of soil aging and other bioavailability factors discussed above (Section 6.3.2), in combination with differing species assemblages and biological accessibility within prey items, ecosystems may also differ in their sensitivity and vulnerability to Pb. The 2006 Pb AQCD reviewed many of these factors which are updated herein by reference to recent literature.

6.3.9.1 Species Sensitivity

There is wide variation in sensitivity of terrestrial species to Pb exposure, even among closely related organisms. Langdon et al. (2005) showed a two-fold difference in LC_{50} values among three common earthworm species, with the standard laboratory species, *E. andrei*, being the least sensitive. Mammalian NOEC values expressed as blood Pb levels were shown to vary by a factor of 8, while avian blood NOECs varied by a factor of 50 (Buekers et al., 2009). Age at exposure, in particular, may affect sensitivity to Pb. For instance, earlier instar *C. elegans* were more likely than older individuals to exhibit neurobehavioral toxicity following Pb exposure (Xing et al., 2009b), and also demonstrated more pronounced neural degeneration than older larvae and adults (Xing et al., 2009c).

6.3.9.2 Nutritional Factors

Dietary factors can exert significant influence on the uptake and toxicity of Pb in many species of birds and mammals. The 2006 Pb AQCD (U.S. EPA, 2006b) describes how Ca^{2+}, Zn, Fe, vitamin E, Cu, thiamin, P, Mg, fat, protein, minerals, and ascorbic acid dietary deficiencies increase Pb absorption and its toxicity. For example, vitamin E content was demonstrated to protect against Pb-induced lipid peroxidation in mallard ducks. Generally, Pb exposure is more likely to produce behavioral effects in conjunction with a nutrient-deficient diet. As previously reported in the 2006 Pb AQCD, Ca^{2+} deficiencies may increase the susceptibility of different terrestrial species to Pb, including plant (Antosiewicz, 2005), avian (Dauwe et al., 2006; Snoeijs et al., 2005) and invertebrate species. Antosiewicz (2005) determined that, for plants, Ca^{2+} deficiency decreased the sequestration capacity of several species (tomato, mustard, rye, and maize),

and that this likely resulted in an increased proportion of Pb at sites of toxic action. Because Pb ions can interact with plant Ca^{2+} channel pores, in the presence of low Ca^{2+} and high Pb concentration, a higher proportion of Pb can interact with these channels and be taken up by plants. A similar phenomenon has been observed in invertebrates, where the metabolic pathway of metals mimics the metabolic pathway of Ca^{2+} [Simkiss et al. (1982), as cited in Jordaens et al. (2006)]. Hence, in environments with disproportionately high Pb versus Ca^{2+} concentration, accumulation of Pb may be accelerated, as in plants. Ca^{2+} deficiency in birds was demonstrated to stimulate the production of Ca^{2+}-binding proteins in the intestinal tract, which extract more Ca^{2+} from available diet; however, this response also enhances the uptake and accumulation of Pb from diet and drinking water [Fullmer (1997), as cited in Dauwe et al. (2006)].

6.3.9.3 Soil Aging and Site-Specific Bioavailability

Total soil Pb concentration is a poor predictor of hazards to avian or mammalian wildlife, because site-specific biogeochemical and physical properties (e.g., pH, OM, metal oxide concentration) can affect the sequestration capacity of soils. Additionally, soil aging processes have been demonstrated to decrease the bioavailable Pb fraction; as such, laboratory toxicity data derived from spiked soils often overestimate the environmental risk of Pb. Smolders et al. (2009) compared the toxicity of freshly Pb-spiked soils to experimentally aged spiked soils and field-collected Pb-contaminated soils. Experimental leaching and aging was demonstrated to increase invertebrate Pb EC_{50} values by factors of 0.4 to greater than 8. In approximately half the cases, experimental aging of freshly spiked soils through leaching removed the proportional relationship between Pb content and toxicity. The leaching/aging factor for Pb, or ratio of ED_{10} values derived in experimentally aged soils to freshly spiked soils, was determined to be 4.2 (factors greater than one indicate decreased toxicity in aged field soils relative to laboratory spiked soils). These results draw attention to the difficulty of extrapolating results from experiments with spiked soil to natural soils, but nonetheless suggest that the sensitivity of terrestrial invertebrates to environmental Pb exposures is likely heavily dependent on the relative rate of aging and site-specific bioavailability.

6.3.9.4 Ecosystem Vulnerability

Relative vulnerability of different terrestrial ecosystems to effects of Pb can be inferred from the information discussed above on species sensitivity and how soil geochemistry influences the bioavailability and toxicity of Pb. Soil ecosystems with low pH, particularly those with sandy soils, are likely to be the most sensitive to the effects of Pb.

Examples of such systems are forest soils, including oak, beech, and conifer forests. The Pine Barrens in southern New Jersey (also known as the Pinelands) is an example of a highly vulnerable ecosystem: it is a dense coniferous (pine) forest with acidic, sandy, nutrient poor soil. As agricultural areas are taken out of production and revert to old fields and eventually forests, their vulnerability to Pb is likely to increase as a result of decreasing OM and acidification of soils (from discontinuation of fertilizing and liming). On the other hand, increasing density of native or invasive plants with associated arbuscular mycorrhizal fungi will likely act to ameliorate some of the effects of Pb (see previous discussion of studies by Sudova and Vostka (2007) and Wong et al. (2007). It is, however, difficult to categorically state that certain plant or soil invertebrate communities are more vulnerable to Pb than others, as the available toxicity data have not yet been standardized for differences in bioavailability (because of use of different Pb salts, different soil properties, and different lengths of aging of soil prior to testing), nutritional state, or organism age, or other interacting factors. Data from field studies are complicated by the co-occurrence of other metals and alterations of pH, such as acidification from SO_2 in smelter emissions, which are almost universal at sites of high Pb exposure, especially at mine or smelter sites. However, because plants primarily sequester Pb in the roots, uptake by soil invertebrates is the most likely pathway for Pb exposure of higher trophic level organisms. Invertivores are likely at higher risk than herbivores. In fact, estimations of Pb risk at a former Pb smelter in northern France indicated that area Pb concentration presented the greatest threat to insectivorous bird and mammal species, but only minimal risk to soil invertebrate and herbivorous mammals (Fritsch et al., 2010). By extension, birds and mammals in ecosystems with a richer biodiversity of soil invertebrates may be more vulnerable to Pb than those in ecosystems with fewer invertebrates (e.g., arid locations). Regardless, the primary determinant of terrestrial ecosystem vulnerability is soil geochemistry, notably pH, CEC, and amount of OM.

6.3.10 Ecosystem Services Associated with Terrestrial Systems

Pb deposited on the surface of, or taken up by organisms has the potential to alter the services provided by terrestrial biota to humans. There are no publications at this time that specifically focus on the ecosystem services affected by Pb in terrestrial systems and the directionality of impacts is not always clear. For example, terrestrial soils provide a service to aquatic ecosystems by sequestering Pb through sorption and precipitation. At the same time, the sequestration of Pb by soils may result in a degredation in the quality of soil and may result in decreased crop productivity. The evidence reviewed in the present document illustrates that Pb can cause ecological effects in each of the four main

categories of ecosystem services (Section 6.1.2) as defined by Hassan et al. (2005). These effects are sorted into ecosystem services categories and summarized here:

- Supporting: altered nutrient cycling, decreased biodiversity, decline of productivity, food production for higher trophic levels
- Provisioning: plant yields
- Regulating: decline in soil quality, detritus production
- Cultural: ecotourism and cultural heritage values related to ecosystem integrity and biodiversity, impacts to terrestrial vertebrates.

A few studies since the 2006 Pb AQCD, consider the impact of metals in general on ecosystem services. Honeybees are important for provisioning services such as pollination and production of honey. They can be exposed to atmospheric Pb by direct deposition or through Pb associated with plants, water or soil. In a study of heavy metals in honeybees in central Italy, there was a statistically significant difference in Pb between bees collected in wildlife reserves compared to bees collected in urban areas with the highest concentration of Pb detected from bees caught in hives near an airport (Perugini et al., 2011). In a review of the effects of metals on insect behavior, ecosystem services provided by insects such as detritus reduction and food production for higher trophic levels were evaluated by considering changes in ingestion behavior and taxis (Mogren and Trumble, 2010). Pb was shown in a limited number of studies to affect ingestion by insects. Crickets (Chorthippus spp) in heavily contaminated sites reduced their consumption of leaves in the presence of increasing Cd and Pb concentrations (Migula and Binkowska, 1993). Decreased feeding activity in larval and adult Colorado potato beetle (*Leptinotarsa decemlineata*) were observed as a result of dietary exposures of Pb and Cu (Kwartirnikov et al., 1999), while no effects were found in ingestion studies of Pb with willow leaf beetle, *Lochmaea caprae* (Rokytova et al., 2004) mottled water hyacinth weevil, *Neochetina eichhorniae* (Kay and Haller, 1986) and hairy springtail, *Orchesella cincta (*van Capelleveen et al., 1986*)*.

Soil health for agricultural production and other soil-associated ecosystem services is dependent upon the maintenance of four major functions: carbon transformations, nutrient cycles, soil structure maintenance, and the regulation of diseases and pests and these parameters may be altered by metal deposition (Kibblewhite et al., 2008). Pb impacts to terrestrial systems reviewed in the previous sections provide evidence for impacts to supporting, provisioning, and regulating ecosystem services provided by soils. For example, earthworms were shown to impact soil metal mobility and availability, which in turn resulted in changes to microbial populations (biodiversity), pH, dissolved organic carbon, and metal speciation (Sizmur and Hodson, 2009), all of which may directly affect soil fertility.

Pb is bioaccumulated in plants, invertebrates and vertebrates inhabiting terrestrial and aquatic systems that receive Pb from atmospheric deposition. This represents a potential route for Pb mobilization into the food web or into food products. For example, Pb bioaccumulation in leaves and roots of an edible plant may represent an adverse impact to the provisioning of food, an essential ecosystem service. Although there is no consistent evidence of trophic magnification there is substantial evidence of trophic transfer. It is through consumption of Pb-exposed prey or Pb-contaminated food that atmospherically deposited Pb reaches species that may have very little direct exposure to it.

There is limited evidence of Pb impacts to plant productivity. Productivity of gray birch (*Betula populifolia*) was impaired in soils with elevated As, Cr, Pb, Zn and V (Gallagher et al., 2008). Tree growth measured in both individuals and at the assemblage level using satellite imagery and field spectrometry was significantly decreased with increasing metal load in soil.

6.3.11 Synthesis of New Evidence for Pb Effects in Terrestrial Systems

This synthesis of the effects of Pb on terrestrial ecosystems covers information from the publication of the 2006 Pb AQCD to present. It is followed in Section 6.5 by determinations of causality that take into account evidence dating back to the 1977 Pb AQCD.

High concentrations of Pb

The state-level mean concentration of Pb in U.S. soils ranges from 5 to 39 mg Pb/kg. Studies of the effects of Pb always include much higher concentrations, whether they use soils that have been exposed to Pb pollution, or experimental amendment with salts of Pb (Table 6-4). Studies that use soils that have been exposed to Pb pollution were conducted in situ, or with soil collected from natural environments in highly contaminated areas near stationary sources. All of them took advantage of gradients of exposure produced by distance from the source to vary the levels of Pb. In some cases, there were only two levels–control and elevated–created by using two sampling locations rather than a larger set of levels representative of the entire gradient. In most studies that used soils exposed to Pb pollution, the highest concentration of Pb in the study is very high relative to those found anywhere except at heavily exposed sites.

In amendment experiments, variation in Pb was generated by addition of Pb salts to either natural or artificial soils. These experiments often included concentrations that were even higher than those found in heavily exposed natural environments. In either type of study,

however, effects gradually increased with increasing exposure. This exposure-response dependency is an important component of causality determinations (Preamble). In addition, until the presence of a discontinuity or breakpoint in the exposure-response relationship can be shown, evidence of a monotonic rise in response suggest that effects are present at all concentrations, even though the magnitude of those effects may not be known in the lower part of the range.

Using concentration-response models where concentration is taken as a continuous variable to analyze data with multiple values of Pb concentration would allow better estimation of the size of effects at any value of exposure, including low ones, and also better estimation of uncertainties around the size of effects. However, none of studies with multiple Pb concentrations used a continuous model to characterize the relationship between concentration and effects. In the analyses of data where multiple levels of Pb were recorded, published studies only used discrete models of exposure, where increasing concentrations of Pb are treated as separate, categorical values.

Comparability of effect concentrations

Strong interactions of Pb concentration and several other soil variables, including pH, CEC, OC, and Fe/Al oxides have been amply demonstrated with respect to various biological responses. For example, Dayton et al.(2006) and Bradham et al.(2006) tested an array of different soils to which the same amount of Pb was added, using lettuce and earthworms, respectively. They found differences in biological effects that were as large as 27, 35, or even 72-fold between soils.

In studies where Pb was introduced through amendment, those interacting variables can be changed experimentally in a controlled way, or held constant. In studies where natural soils were used in which Pb originated from pollution, they are left to vary freely. In either case, the presence and magnitude of those interactions make calculations of expected responses under other sets of conditions particularly difficult, as well as comparisons between studies conducted under different conditions.

In addition, the amount of Pb dissolved in soil pore water determines the impact of soil Pb on terrestrial ecosystems to a much greater extent than the total amount present. It has long been established that the amount of Pb dissolved in soil solution is controlled by at least six variables: (1) solubility equilibria; (2) adsorption-desorption relationship of total Pb with inorganic compounds; (3) adsorption-desorption reactions of dissolved Pb phases on soil OM; (4) pH; (5) CEC; and (6) aging. Since 2006, further details have been contributed to the understanding of the role of pH, CEC, OM, and aging. Smolders et al. (2009) demonstrated that the two most important determinants of solubility (and also

toxicity) in soils are pH and CEC. However, they had previously shown that aging, primarily in the form of initial leaching following deposition, decreases soluble metal fraction by approximately one order of magnitude (Smolders et al., 2007). Since 2006, OM has been confirmed as an important influence on Pb sequestration, leading to longer-term retention in soils with higher OM content, and also creating the potential for later release of deposited Pb. Aging, both under natural conditions and simulated through leaching, was shown to substantially decrease bioavailability to plants, microbes, and vertebrates. However, most studies report some measure of total extracted Pb, or total added Pb, rather than pore water or soluble fraction.

Plants

Recent studies with herbaceous species growing at various distances from smelters added to the existing strong evidence that atmospherically transported Pb is taken up by plants. These studies did not establish the relative proportion that originated from atmospheric Pb deposited in the soil, as opposed to that taken up directly from the atmosphere through the leaves. Studies found that in trees, Pb that is taken up through the roots is then generally translocated from the roots to other parts. However, multiple recent studies showed that in trees, the proportion of Pb that is taken up through the leaves is likely to be very substantial. One study attempted to quantify it, and suggested that 50% of the Pb contained in Scots Pine in Sweden is taken up directly from the atmosphere (Section 6.3.3.1). Studies with herbaceous plants found that in most species tested, soil Pb taken up by the roots is not translocated into the stem and leaves, but when growth and survival were reported, growth of the whole plant decreased with increasing Pb, and mortality increased (Table 6-4). Experimental studies have added to the existing evidence of photosynthesis impairment in plants exposed to Pb, and have found damage to photosystem II due to alteration of chlorophyll structure, as well as decreases in chlorophyll content in diverse taxa, including lichens and mosses. A substantial amount of evidence of oxidative stress in response to Pb exposure has also been produced. Reactive oxygen species were found to increase in broad bean and tomato plants exposed to increasing concentrations of soil Pb, and a concomitant increase in superoxide dismutase, glutathione, peroxidases, and lipid peroxidation, as well as decreases in catalase were observed in the same plants. Monocot, dicot, and bryophytic taxa grown in Pb-contaminated soil or in experimentally spiked soil all responded to increasing exposure with increased antioxidant activity. In addition, genotoxicity, decreased germination, and pollen sterility were observed in some experiments. All effects were small outside of contaminated areas (Section 6.3.4.1).

Invertebrates

Since the 2006 Pb AQCD, various species of terrestrial snails have been found to accumulate Pb from both diet and soil, although effects on growth, survival and reproduction are inconsistent. Recent studies with earthworms have found that both internal concentration of Pb and mortality increase with decreasing soil pH and CEC. In addition, tissue concentration differences have been found in species of earthworms that burrow in different soil layers. The rate of accumulation in each of these species could result from layer differences in interacting factors such as pH and CEC (Section 6.3.3.2). Because earthworms often sequester Pb in granules, some authors have suggested that earthworm Pb is not bioavailable to their predators. There is some evidence that earthworm activity increases Pb availability in soil, but it is inconsistent. In arthropods collected at contaminated sites, recent studies found gradients in accumulated Pb that corresponded to gradients in soil with increasing distance from stationary sources. Recently published studies have shown neuronal damage in nematodes exposed to low concentrations of Pb (2.5 μM = 0.5 mg Pb/L), accompanied by behavioral abnormalities. Reproductive effects were found at lower exposure in younger nematodes, and effects on longevity and fecundity were shown to persist for several generations. Increased mortality was found in earthworms, and was strongly dependent on soil characteristics including pH, CEC, and aging. Snails exposed to Pb through either topical application or through consumption of Pb-exposed plants had increased antioxidant activity and decreased food consumption, but effects on growth and survival were inconsistent. Effects on arthropods exposed through soil or diet varied with species and exposure conditions, and included diminished growth and fecundity in springtails, endocrine and reproductive anomalies, and body deformities. Increasing concentration of Pb in the exposure medium generally resulted in increased effects within each study, but the relationship between concentration and effects varied between studies, even when the same medium, e.g., soil, was used (Section 6.3.4.2). Evidence suggested that aging and pH are important modifiers.

Vertebrates

There were few recent studies of Pb bioavailability and uptake in vertebrates since the 2006 Pb AQCD. A study of two species of sea ducks in Alaska found that 3% of the birds had tissue levels of Pb that indicated exposure above background. Urban pigeons in Korea were found to accumulate 1.6 to 1.9 mg Pb/kg wet weight Pb in the lungs, while in Wisconsin 70% of American woodcock chicks and 43% of young-of-year had elevated bone Pb (9.6 to 93 mg Pb/kg dry weight in chicks, 1.5 to 220 mg Pb/kg dry weight in young-of-year). None of the locations for these studies was in proximity to stationary sources of heavy contamination, and none was able to identify the origin of the Pb

(Section 6.3.3.3). Effects on amphibians and reptiles included decreased white blood cell counts, decreased testis weight, and behavioral anomalies. However, large differences in effects were observed at the same concentration of Pb in soil, depending on whether the soil was freshly amended or field-collected from contaminated areas. As in most studies where the comparison was made, effects were smaller when field-collected soils were used. A study at the Anaconda Smelter Superfund site found increasing Pb accumulation in gophers with increasing soil Pb around the location of capture. Effects of dietary exposure were studied in several mammalian species, and cognitive, endocrine, immunological, and growth effects were observed. Pigs fed various Pb-contaminated soils showed that the form of Pb determined accumulation, and another study showed lower feed efficiency and weight in pigs with 2.08 versus 1.44 μg Pb/dL in blood, originating in Pb-sulfate feed supplement. In some birds, maternal elevated blood Pb level was associated in recent studies with decreased hatching success, smaller clutch size, high corticosteroid level, and abnormal behavior. Some species show little or no effect of elevated blood Pb level. A study of Japanese quail found that Pb added to the diet could improve survival and incidence of several pathologies, and a long term study of pied flycatchers at a mine site produced mixed evidence for the effects of Pb (Section 6.3.4.3).

Food web

Recent studies were able to measure Pb in the components of various food chains that included soil, plants, invertebrates, arthropods and vertebrates. They confirmed that trophic transfer of Pb is pervasive, but no consistent evidence of trophic magnification was found (Section 6.3.3.4).

Community and Ecosystem Effects

New evidence of effects of Pb at the community and ecosystem levels of biological organization include several studies of the ameliorative effects of mycorrhizal fungi on plant growth, attributed to decreased uptake of Pb by plants, although both mycorrhizal fungus and plant were negatively affected. The presence of both earthworms and mycorrhizal fungi decreased solubility and mobility of Pb in soil in one study, but the presence of earthworms was associated with higher uptake of Pb by plants in another. The presence of snails increased uptake of Pb by earthworms, but not vice-versa. Most recently published research on community and ecosystem effects of Pb has focused on soil microbial communities, which have been shown to be impacted in both composition and activity. Many recent studies have been conducted using mixtures of metals, but have tried to separate the effects of individual metals when possible. One study compared the

effects of 6 metals individually (Åkerblom et al., 2007), and found that their effects on community composition were similar. In studies that included only Pb, or where effects of Pb could be separated, soil microbial activity was generally diminished, but in some cases recovered over time. Species and genotype composition were consistently altered, and those changes were long-lasting or permanent (Section 6.3.6).

Exposure-Response

Several studies with various organisms have included gradients of Pb exposure. None has characterized the exposure-relationship using a continuous model of exposure-response. However, evidence indicates clearly that increased exposure to Pb is associated with increases in observed effects in terrestrial ecosystems. Evidence also demonstrates that many factors, including species and various soil physiochemical properties, interact strongly with Pb concentration to modify those effects. In terrestrial ecosystems, where soil is generally the main component of the exposure route, Pb aging is a particularly important factor, and one that may be difficult to reproduce experimentally. Without adequate quantification of those interactions, characterizations of exposure-response relationships may be difficult to transfer outside of experimental settings.

6.3.12 Causal Determinations for Pb in Terrestrial Systems

In the following sections, organism-level effects on reproduction and development, growth and survival are considered first since these endpoints can lead to effects at the population level or above and are important in ecological risk assessment. Neurobehavioral effects are considered next followed by sub-organismal responses (hematological effects, physiological stress) for which Pb has been shown to have an impact in multiple species and across taxa, including humans. Causal determinations for terrestrial, freshwater and saltwater ecological effects are summarized in Table 6-3.

6.3.12.1 Reproductive and Developmental Effects-Terrestrial Biota

In terrestrial invertebrates and vertebrates, evidence assessed for the present document and in Pb AQCDs indicates an association between reproductive effects and Pb exposure. Impaired fecundity at the organism level of biological organization can result in a decline in abundance and/or extirpation of populations, decreased taxa richness, and decreased relative or absolute abundance at the community level (Suter et al., 2005; U.S. EPA, 2003a). Evaluation of the literature on Pb effects in terrestrial species indicates that exposure to Pb is associated with reproductive effects. Various endpoints have been

measured in various taxa of terrestrial organisms to assess the effect of Pb on fecundity, development, and hormone homeostasis. Although reproductive effects were demonstrated, no single endpoint in a single taxon has been extensively studied. Recent evidence available since the 2006 Pb AQCD for effects of Pb on reproductive endpoints in terrestrial species is summarized in Table 6-4.

In terrestrial plants, few studies were available to the 2006 Pb AQCD (U.S. EPA, 2006b), and few are available more recently that specifically address reproductive effects of Pb exposure. Two genotypes of maize seedlings exhibited a significant and concentration-dependent reduction in seed germination following 7 days of Pb treatment in nutrient solution of nominal concentration of 0, 0.007, 0.7 and 7 mg Pb/L as Pb sulfate (Ahmad et al., 2011). Germination inhibition and chromosomal abnormalities also increased in a concentration-dependent manner in Grass pea grown in soil irrigated with solutions containing nominal concentration of 0 to 188 mg Pb/L (Kumar and Tripathi, 2008). However, germination increased in a broad sample of soils when amended with 2,000 mg Pb/kg (Dayton et al., 2006).

In terrestrial invertebrates, Pb can alter developmental timing, hatching success, sperm morphology and hormone homeostasis. The number of species studied has been small, but reproductive effects consistently increase with increasing exposure. The 2006 Pb AQCD reported effects on reproduction in collembolans and earthworms, with LOECs and NOECs typically well above Pb soil concentrations observed away from stationary sources of contamination. more recently, an increase in development time (approximately two days) and a reduction in relative fecundity were observed in aphids feeding on plants exposed to high concentrations of Pb (Görür, 2007). Hatching success of the collembolan *F. candida* was decreased following 10 day exposure to Pb-spiked soils (EC_{50} 2,361 mg Pb/kg dry soils) (Xu et al., 2009b). Sperm morphology in Asian earthworms was significantly altered following 2-week exposures to soils containing nominal concentration of 1,000, 1,400, 1,800, and 2,500 mg Pb/kg soil (Zheng and Li, 2009). Pb may also disrupt hormonal homeostasis in invertebrates as studies with moths have suggested (Shu et al., 2009). Adult female moths reared on diets containing 25, 50, 100, or 200 mg Pb/kg exhibited decreased vitellogenin mRNA induction, and vitellogenin levels were demonstrated to decrease with increasing Pb exposure. Evidence of multi-generational toxicity effects of Pb is also present in terrestrial invertebrates, specifically springtails, mosquitoes, carabid beetles, and nematodes where decreased fecundity in progeny of Pb-exposed individuals was observed. The magnitude of effects is variable, but they are present in multiple phyla, and increase with increasing exposure within studies. Reproductive effects in terrestrial invertebrates are also coherent with similar effects observed in aquatic invertebrates.

In terrestrial vertebrates, there is evidence for reproductive effects associated with Pb exposure in recent evidence and Pb AQCDs. The 2006 Pb AQCD (U.S. EPA, 2006c) concluded that exposure to affects reproduction and development in terrestrial vertebrates. Effects reported in that document included declines in clutch size, number of young hatched, number of young fledged, decreased fertility, and decreased eggshell thickness observed in birds near areas of Pb contamination and in birds with elevated Pb tissue concentration regardless of location. More recently, decreased testis weight was observed in lizards administered a sublethal dose of 10 or 20 mg Pb/kg day by oral gavage for 60 days (Salice et al., 2009). Few studies in the field have addressed reproductive effects of Pb specifically in mammals, due to most available data in wild or grazing animals being from near smelters, where animals are co-exposed to other metals. For example, the reproductive viability of red deer (*C. elaphus*) inhabiting a Pb-contaminated mining area of Spain was shown to be altered, with 11% and 15% reductions in spermatozoa and acrosome integrity observed in male deer from the mining area compared with those residing in reference areas (Reglero et al., 2009a), but multiple other metals were present at high concentrations. Evidence from AQCDs and the present document for terrestrial vertebrates is coherent with evidence from freshwater amphibians, and fish (Section 6.4.12.1). However, experimental evidence obtained using mammals in the context of human health research demonstrates a consistency of adverse effects of Pb on sperm (Section 4.8.3.1) and the onset of puberty in males and females (Sections 4.8.1.1 [females] and 4.8.1.2 [males]) with strong evidence from both toxicology and epidemiology studies. Other reproductive endpoints including spontaneous abortions, pre-term birth, embryo development, placental development, low birth weight, subfecundity, hormonal changes, and teratology were also affected, but less consistently (Section 4.8).

For reproductive and developmental effects in terrestrial ecosystems, the current body of evidence is inadequate to conclude that exposure to Pb is causal in plants, and is sufficient to conclude that there is a causal relationship in invertebrates and vertebrates.

6.3.12.2 Growth Effects-Terrestrial Biota

Alterations in growth at the organism level of biological organization can have impacts at the population, community, and ecosystem levels. In terrestrial ecosystems, evidence for effects of Pb on growth is strongest in terrestrial plants, although these effects are typically observed in laboratory studies with high exposure concentrations or in field studies near stationary sources. In terrestrial plants, there is evidence over several decades of research that Pb inhibits photosynthesis and respiration, all of which can reduce the growth of the plant (U.S. EPA, 2006c, 1986a, 1977). Decreases in chlorophyll *a* and *b*

content have been observed in various algal and plant species. Many laboratory toxicity studies report effects on the growth of plants in synthetic growing media, but observed effects typically occur at concentrations higher than the average background concentrations in U.S. soils (19 mg Pb/kg dry weight) (U.S. EPA, 2005b) and there are few field studies. Effects on plant growth can result in reduced productivity and decreased biomass. The 2006 Pb AQCD relied principally on evidence assembled in the *Ecological Soil Screening Levels for Lead* document (U.S. EPA, 2005b), which concluded that growth (biomass) was the most sensitive and ecologically relevant endpoint for plants.

Evidence for growth effects in terrestrial fauna is sparse. In the 1986 Pb AQCD, a study was reviewed in which the F1 and F2 generations of the springtail *Onychiurus armatus* fed a diet of Pb-exposed fungi (0.008 to 3.1 mg Pb/g) experienced a delay in achieving maximum length (Bengtsson et al., 1983). The authors suggested that the reduced growth may be accompanied by delayed sexual maturity. The 2006 Pb AQCD (U.S. EPA, 2006b) reported that growth effects observed in both terrestrial invertebrates and vertebrates were more pronounced in juvenile organisms, underscoring the importance of lifestage to overall Pb susceptibility. Recent evidence available since the 2006 Pb AQCD for effects of Pb on growth endpoints in terrestrial species is summarized in Table 6-4: reduced growth of garden snail *T. pisana*, increasing with increasing exposure, was observed following a five week dietary exposure to eight nominal concentrations of Pb (El-Gendy et al., 2011). Studies also show concentration-dependent inhibition of growth in earthworms raised in Pb-amended soil (Zheng and Li, 2009; Currie et al., 2005; Langdon et al., 2005). In AQCDs, growth effects of Pb have been reported in birds (changes in juvenile weight gain), at concentrations typically higher than currently found in the environment away from heavily exposed sites. The current body of evidence is sufficient to conclude that there is a causal relationship between Pb exposures and growth effects in terrestrial plants, and that a causal relationship is likely to exist between Pb exposure and growth effects in terrestrial invertebrates. Evidence is inadequate to establish causal relationship between Pb exposures and growth effects in terrestrial vertebrates.

6.3.12.3 Survival-Terrestrial Biota

The relationship between Pb exposure and survival has been well demonstrated in terrestrial species as presented in the Pb AQCDs and in Section 6.3.5 of the present document. Exposure can be either lethal, or produce sublethal effects that diminish survival probabilities. In the 1977 Pb AQCD, deaths from Pb poisoning in domestic animals caused by emissions from stationary sources were reported (U.S. EPA, 1977).

Additional studies in the 1986 and 2006 Pb AQCDs and current ISA provide evidence for a concentration-dependent response of mortality in terrestrial biota. Recent evidence available since the 2006 Pb AQCD for effects of Pb on survival in terrestrial species is summarized in Table 6-4.

Survival is a biologically important response that can have direct impact on population size. Survival is often quantified using LC_{50} (the concentration of toxicant where 50% mortality is observed or modeled), which may be a better metric for acute toxicity than for typical environmental exposure, which is more often comparatively low, and cumulative or chronic. From the LC_{50} data on Pb in this review and previous Pb AQCDs, a wide range of sensitivity to Pb is evident across taxa and within genera. As expected, reported LC_{50} are usually much higher than current environmental levels of Pb in the U.S away from heavily exposed sites, even though physiological dysfunction that adversely impacts the fitness of an organism often occurs well below concentrations that result in mortality. When available, LC_{10}, NOEC, or LOEC have been reported in the present document.

Pb is generally not phytotoxic to plants at concentrations found in the environment away from heavily exposed sites, probably due to the fact that plants often sequester large amounts of Pb in roots, and that translocation to other parts of the plant is limited. No data have become available to change this assessment since the 2006 Pb AQCD.

Survival of soil-associated organisms is adversely affected by Pb exposure. In the 1986 Pb AQCD it was reported that Pb at the high extreme of concentrations found near roadsides and smelters at the time (10,000 to 40,000 mg Pb/kg dry weight) can eliminate populations of bacteria and fungi on leaf surfaces and in soil. Severe impairment of decomposition has long been accepted to be one of the most apparent results of soil contamination with Pb and other metals. In nematodes, the 2006 Pb AQCD reported LC_{50} values varying from 10 to 1,550 mg Pb/kg dry weight dependent upon soil OM content and soil pH (U.S. EPA, 2006c). In earthworms, 14 and 28 day LC_{50} values typically fell in the range of 2,400-5,800 mg Pb/kg depending upon the species tested. More recent evidence has been consistent with these values, and also showed concentration-dependent decreases in survival in collembolans and earthworms under various experimental conditions. The evidence in terrestrial invertebrates is coherent with evidence in freshwater invertebrates.

In terrestrial avian and mammalian species, toxicity is observed in laboratory studies over a wide range of doses (<1 to >1,000 mg Pb/kg body weight per day) as reviewed for the development of Eco-SSLs (U.S. EPA, 2005b), and subsequently reported in the 2006 Pb AQCD. The NOAELs for survival ranged from 3.5 to 3,200 mg Pb/kg per day. Surprisingly, the only study to have reported survival data following exposure to Pb in an

avian species since the 2006 Pb AQCD, found that survival was greater than in controls in quail exposed to 50 mg Pb/L in drinking water for 7 weeks (Nain and Smits, 2011). Evidence for association of Pb exposure with mortality in terrestrial vertebrates is coherent with observations in freshwater vertebrates (Section 6.4.12.3). Therefore, the evidence is sufficient to conclude that a causal relationship is likely to exist between Pb exposures and survival in terrestrial vertebrates and that there is a causal relationship between Pb exposures and survival in terrestrial invertebrates. The evidence is inadequate to conclude that there is a causal relationship between Pb exposures and survival in terrestrial plants.

6.3.12.4 Neurobehavioral Effects-Terrestrial Biota

The central nervous system of animals was recognized as a target of Pb toxicity in the 1977 Pb AQCD (U.S. EPA, 1977), and subsequent Pb reviews have provided additional supporting evidence of Pb as a neurotoxicant in terrestrial invertebrates and vertebrates. Effects of Pb on neurological endpoints in terrestrial animal taxa include changes in behaviors that may decrease the overall fitness of the organism such as food consumption, prey capture ability and avoidance behaviors.

Some organisms exhibit behavioral avoidance while others do not seem to detect the presence of Pb (U.S. EPA, 2006c). Decreased food consumption of Pb-contaminated diet has been demonstrated in some invertebrates (snails) and vertebrates (lizards, pigs). Decreased food consumption was observed in juvenile *A. achatina* snails exposed to Pb-contaminated (concentration greater than 134 mg Pb/kg) diet for 12 weeks (Ebenso and Ologhobo, 2009a). Similarly, feeding rate in *T. pisana* snails was depressed in 3 week dietary exposures of 50 to 15,000 mg Pb/kg (El-Gendy et al., 2011), while other snails exposed to Pb at similar concentrations have shown no effects on feeding rate (Beeby and Richmond, 2010). Consumption of 10 mg/Pb kg diet resulted in lower food intake after 120 days of dietary exposure in pigs (*S. domestica*) (Yu et al., 2005).

In limited studies available on nematodes there is evidence that Pb may affect the ability to escape or avoid predation (Wang and Xing, 2008). Additional new evidence of changes in the morphology of GABA motor neurons was also found in nematodes (*C. elegans*) (Du and Wang, 2009).

Gull chicks experimentally exposed to Pb exhibited abnormal behaviors such as decreased walking, learning deficits, erratic behavioral thermoregulation, and food begging that could make them more vulnerable in the wild (Burger and Gochfeld, 2005). Pb was administered via injection to reach a Pb concentration in feathers equivalent to Pb levels in feathers of wild gull populations. Lizards exposed to Pb through diet of 10 to

20 mg Pb/kg per day for 60 days in the laboratory exhibited abnormal coloration and posturing behaviors.

These findings in terrestrial invertebrates and vertebrates are coherent with findings from studies in aquatic biota that showed neurobehavioral alterations in various species of fish, and also in some aquatic invertebrates (Section 6.4.12.4). They are also coherent with findings in laboratory animals that show that Pb induces changes in learning and memory (Section 4.3.2.3). New behaviors induced by exposure to Pb reviewed in Chapter 4 that are relevant to effects of Pb observed in terrestrial systems include hyperactivity and mood disorders, effects on visual and auditory sensory systems, changes in structure and function of neurons and supporting cells in the brain, and effects on the blood brain barrier. Mechanisms that include the displacement of physiological cations, oxidative stress and changes in neurotransmitters and receptors are also reviewed. Data from ecological studies are highly coherent with these data from animal experiments, especially neurobehavioral findings and evidence of structural changes. Overall, the evidence from aquatic and terrestrial systems is sufficient to conclude that a causal relationship is likely to exist between Pb exposures and neurobehavioral effects in invertebrates and vertebrates in terrestrial ecosystems.

6.3.12.5 Hematological Effects-Terrestrial Biota

Hematological responses are commonly reported effects of Pb exposure in vertebrates in terrestrial systems. In the 1977 Pb AQCD, ALAD was recognized as the most sensitive indicator of Pb exposure in rats (U.S. EPA, 1977). Furthermore, inhibition of ALAD was associated with death of waterfowl following ingestion of Pb shot. In the 1986 Pb AQCD, decreases in red blood cell ALAD activity were documented in birds and mammals near a smelter (Beyer et al., 1985). Additional evidence for effects on blood parameters and their applicability as biomarkers of Pb exposure in terrestrial birds and mammals were presented in the 2005 *Ecological Soil Screening Levels for Lead*, the 2006 Pb AQCD and the current ISA (U.S. EPA, 2006c, 2005b). Field studies available since the 2006 Pb AQCD, include evidence for elevated blood Pb levels correlated with decreased ALAD activity in songbirds and owls living in historical mining areas (Gómez-Ramírez et al., 2011; Hansen et al., 2011a).

This evidence is strongly coherent with evidence from freshwater invertebrates and vertebrates (Section 6.4.12.5) and observations from human epidemiologic and animal toxicology studies showing that exposure to Pb induces effects on hematological endpoints, including altered heme synthesis mediated through decreased ALAD and ferrochelatase activities, decreased red blood cell survival and function, and increased red

blood cell oxidative stress. Taken together, the overall weight of human epidemiologic and animal toxicological evidence is sufficient to conclude that a causal relationship exists between Pb exposure and decreased RBC survival and function, and altered heme synthesis in humans and in laboratory animals (Section 4.7). Based on observations in terrestrial birds and mammals and additionally supported by observations in aquatic organisms, and toxicological and epidemiological findings in laboratory animals and humans evidence is sufficient to conclude that there is a causal relationship between Pb exposures and hematological effects in terrestrial vertebrates. The evidence is inadequate to conclude that there is a causal relationship between Pb exposures and hematological effects in terrestrial invertebrates.

6.3.12.6 Physiological Stress-Terrestrial Biota

Induction of enzymes associated with oxidative stress response in terrestrial plants, invertebrates and vertebrates is a recognized effect of Pb exposure (U.S. EPA, 2006c). Several studies from the 2006 Pb AQCD in birds and plants provide evidence that Pb induces lipid peroxidation, however, exposures in these studies were higher than would be found generally in the environment (U.S. EPA, 2006c). Building on the body of evidence presented in the 2006 Pb AQCD, recent studies provide evidence of upregulation of antioxidant enzymes and increased lipid peroxidation associated with Pb exposure in many species of plants, invertebrates and vertebrates. In plants, increases of antioxidant enzymes with Pb exposure occur in some terrestrial species at concentrations approaching the average Pb concentrations in U.S. soils (18.9 mg Pb/kg). For example, in a series of studies Wang et al. observed increases in reactive oxygen species with increasing exposure to Pb from 20 mg Pb/kg soil to 2,000 mg Pb/kg in broad bean (*V. faba*) (Wang et al., 2010c; Wang et al., 2010a; Wang et al., 2008b) and tomato (*L. esculentum*) (Wang et al., 2008a), where they were accompanied up to approximately 500mg Pb/kg by proportional increases in SOD, glutathione, guaiacol peroxidase, and lipid peroxidation, as well as decreases in catalase. Spinach seedlings grown in soil containing six increasing concentrations of Pb from 20 to 520 mg Pb/kg exhibited higher production of reactive oxygen species, increased rates of lipid peroxidation and increased SOD concentrations (Wang et al., 2011a). Markers of oxidative damage are also observed in terrestrial invertebrates, including snails and earthworms, and in terrestrial mammals. Across these biota, there are differences in the induction of antioxidant enzymes that appear to be species-dependent.

The oxidative stress responses associated with Pb exposure in terrestrial plants, invertebrates and vertebrates are consistent with responses in freshwater (Section 6.4.12.6) and saltwater organisms (Section 6.4.21.6), and in humans (Section 4.2.4). This oxidative stress is characterized by increased presence of reactive oxygen species and membrane and lipid peroxidation that can promote tissue damage, cytotoxicity, and dysfunction. Increases in reactive oxygen species are often followed by a compensatory and protective upregulation in antioxidant enzymes, such that this upregulation is itself indicative of oxidative stress conditions. Continuous production of reactive oxygen species may overwhelm this defensive process, leading to further oxidative stress and injury.

Upregulation of antioxidant enzymes and increased lipid peroxidation are considered reliable biomarkers of stress, and provide evidence that Pb exposure induces a stress response in those organisms which may itself increase susceptibility to other stressors and reduce individual fitness. Evidence is sufficient to conclude that there is a causal relationship between Pb exposures and physiological stress in terrestrial plants, and that a causal relationship is likely to exist between Pb exposure and physiological stress in terrestrial invertebrates and vertebrates.

6.3.12.7 Community and Ecosystem Level Effects-Terrestrial Biota

Most direct evidence of community and ecosystem level effects is from near stationary sources where Pb concentrations are higher than typically observed environmental concentrations for this metal. Impacts of Pb on terrestrial ecosystems near smelters, mines, and other industrial sources have been studied for several decades. Emissions of Pb from smelting and other industrial activities are accompanied by other trace metals (e.g., Zn, Cu, Cd) and SO_2 that may cause toxic effects independently or in concert with Pb. Those impacts include decreases in species diversity and changes in floral and faunal community composition. Ecosystem-level field studies are complicated by the confounding of Pb exposure with other factors such as the presence of trace metals and acidic deposition and the inherent variability in natural systems. In natural systems, Pb is often found co-existing with other stressors, and observed effects may be due to cumulative toxicity.

In laboratory and microcosm studies where it is possible to isolate the effect of Pb, this metal has been shown to alter competitive behavior of species, predator-prey interactions and contaminant avoidance. These dynamics may change species abundance and community structure at higher levels of ecological organization. Uptake of Pb into aquatic and terrestrial organisms and subsequent effects on mortality, growth,

physiological stress, blood, neurobehavioral and developmental and reproductive endpoints at the organism level are expected to have ecosystem-level consequences, and thus provide consistency and plausibility for causality in ecosystem-level effects.

In the 1977 Pb AQCD the potential for Pb to interfere with ecosystem level processes was explored in a detailed review of a study on the effects of Pb on relationships between arthropods and leaf litter decomposition (U.S. EPA, 1977). Reduced arthropod density, biomass and richness were observed in the vicinity of a Pb smelting complex in Missouri. There were also several studies correlating feeding habits, habitat, and Pb concentrations in body tissues reported in the 1977 Pb AQCD, specifically in insects and small mammals indicating that species differences in Pb concentrations are determined in part by trophic position and habitat preference.

In the 1986 Pb AQCD it was reported that Pb at environmental concentrations occasionally found near roadsides and smelters (10,000 to 40,000 mg Pb/kg dry weight [mg Pb/kg]) can eliminate populations of bacteria and fungi on leaf surfaces and in soil (U.S. EPA, 1986b). Some key populations of soil microorganisms and invertebrates die off at 1,000 mg Pb/kg soil interrupting the flow of energy through decomposition processes and altering community structure. At soil concentrations of 500 to 1,000 mg Pb/kg or higher, populations of plants, microorganisms, and invertebrates may shift toward Pb-tolerant populations of the same or different species (U.S. EPA, 1986b).

The 2006 Pb AQCD reported that decreased species diversity, changes in floral and faunal community composition and decreased vigor of terrestrial vegetation were observed in ecosystems surrounding former smelters including the Anaconda smelter in southwestern Montana (U.S. EPA, 2006c). Several studies in the 2006 Pb AQCD documented reduced organic matter decomposition rates and decreased microbial biomass in areas heavily polluted by metals. Lower abundance and reduced biodiversity of soil invertebrate communities were observed in field surveys in proximity to Pb stationary sources.

Recent evidence published since the 2006 Pb AQCD (summarized in Table 6-4) supports previous findings of a link between high concentration of soil metals and substantial changes in soil microorganism community composition, as well as decreased abundance and diversity. In a naturally Pb-enriched forest in Norway, The number of fungal colony forming units was approximately 10 times lower in the highest Pb soil concentration (~4.5 mg Pb/g dry weight) than in control soils (Baath et al., 2005). The composition of the fungal community was drastically altered, with only one species common to both soils, and the number of species present was substantially lower.

The effect of Pb on microbial community function has been quantified previously using functional endpoints such as respiration rates, fatty acid production, and soil acid phosphatase and urease activities. These may provide estimates of ecological impacts that emphasize functionality irrespective of microbial diversity or abundance measurements. Studies available since the 2006 Pb AQCD provide further evidence of Pb effects on microbial processes. Pb contamination reduced phenol oxidase activity in several types of soils; concentrations between 5 and 50 nM Pb (0.001 and 0.01 mg Pb/L) significantly decreased phenol oxidase activity in all soils tested, while 400 nM (0.08 mg Pb/L) and greater completely arrested phenol oxidase activity in one soil tested (a high pH sandy loam) (Carine et al., 2009). Pb concentrations between 50 and 500 mg Pb/kg significantly reduced microbial abundance and diversity, and also resulted in lower soil phosphatase, urease, and dehydrogenase activities (Gao et al., 2010b). When the microbial properties of metal-contaminated urban soils were compared to those of rural soils, significant differences (Sudova and Vosatka, 2007) were detected in basal community respiration rates and microbial abundance (Yang et al., 2006). Gai et al. (2011) examined the microbial activity of three soils via microcalorimetric methods following Pb exposure. They noted an increase in activity immediately following Pb application (giving 10, 20, 40, 80, and 160 mg Pb/kg), and theorized that this was a result of rapid mortality of sensitive microbial species, followed by a concurrent proliferation of Pb-tolerant microorganisms. As Pb concentrations increased, however, the calculated microbial growth rate constant decreased, indicating a suppression of microbial activity (Gai et al., 2011). Akerblom et al. (2007) tested the effects of six metals (Cr, Zn, Mo, Ni, Cd, and Pb) individually. All tested metals had a similar effect on the species composition of the microbial community. Exposure to a high Pb concentration (52 mg Pb/kg) negatively affected respiration rates.

In addition to microbial communities, there is new evidence for effects of Pb on other terrestrial ecosystem components. Increased plant diversity was shown to ameliorate effects of Pb contamination on a microbial community (Yang et al., 2007). The presence of arbuscular mycorrhizal fungi may protect plants growing in Pb-contaminated soils (Bojarczuk and Kieliszewska-Rokicka, 2010; Sudova and Vosatka, 2007). Invertebrates affected by Pb in terrestrial systems may be altering community structure. Recent evidence since the 2006 Pb AQCD, indicates that some species of worms avoid Pb-contaminated soils (Langdon et al., 2005). Reductions in microbial and detritivorous populations can affect the success of their predators (U.S. EPA, 2006c). Following a 28-day exposure to field-collected soils contaminated with metals (including Pb at 426 mg Pb/kg), both population growth and individual growth of the earthworm *L. rubellus* were diminished (Klok et al., 2006). The authors proposed that, although these reductions were unlikely to result in extirpation, avian predators such as the godwit (*Limosa limosa*) that feed heavily on earthworms may be affected by a reduction of

available earthworm biomass. Furthermore, the presence of earthworms increased Pb uptake by plants (Ruiz et al., 2011; Sizmur et al., 2011).

In terrestrial ecosystems, most studies show decreases in microorganism abundance, diversity, and function with increasing soil Pb concentrations in areas near point-sources. Specifically, shifts in nematode communities, bacterial species, and fungal diversity have been observed. Most evidence for Pb toxicity to terrestrial plants, invertebrates and vertebrates is from single-species assays in laboratory studies. Although the evidence is strong for effects of Pb on growth (Section 6.3.12.2), reproduction (Section 6.3.12.1) and survival (Section 6.3.12.3) in certain species, considerable uncertainties exist in generalizing effects observed under small-scale, particular conditions up to predicted effects at the ecosystem level of biological organization. In many cases it is difficult to characterize the nature and magnitude of effects and to quantify relationships between ambient concentrations of Pb and ecosystem response due to existence of multiple stressors, variability in field conditions, and to differences in Pb bioavailability at that level of organization. However, the cumulative evidence for Pb effects at higher levels of ecological organization is sufficient to conclude that a causal relationship is likely to exist between Pb exposures and the alteration of species richness, species composition and biodiversity in terrestrial ecosystems.

6.4 Aquatic Ecosystem Effects

6.4.1 Introduction to Effects of Pb on Aquatic Ecosystems

This section of the Pb ISA reviews the recent literature published since the 2006 Pb AQCD (U.S. EPA, 2006c), on the effects of Pb on freshwater and saltwater ecosystems. Freshwater and marine/estuarine systems are considered separately due to differences in Pb speciation, bioavailability of Pb, salinity, and physiological adaptations of organisms in freshwater versus saltwater environments, as modifying factors for Pb toxicity. The focus is on the effects of Pb to aquatic organisms including algae, aquatic plants, invertebrates, vertebrates, and other biota with an aquatic lifestage (e.g., amphibians). In the freshwater and saltwater sections, aqueous concentrations of Pb are reported as µg Pb/L and sediment concentrations are in mg Pb/kg.

In the present document, studies in some freshwater and saltwater organisms are included where responses are observed at very high Pb concentrations that might not be expected in most environmental scenarios or where the relevance of the exposure method to atmospherically-deposited Pb is unknown. These studies can provide mechanistic information on Pb toxicity, allow for comparison of Pb uptake across taxa, or

demonstrate the wide range of sensitivity among closely-related species. Furthermore, although exposure to Pb in natural systems is likely characterized as a chronic, low dose exposure, it is not always feasible to conduct long-term experiments under natural conditions. Observations from short-term experiments in which high concentrations are used can help to elucidate the shape of concentration-response relationships and provide evidence for a gradient of response to Pb exposure but the extent to which effects would be observed at concentrations of Pb typically found in the environment is uncertain.

There are a few studies in the following sections for which effects are reported at very low concentrations of Pb that appear to be below analytical detection limits. These studies are included to the extent that they provide information on responses to Pb. However, the difficulty in maintaining low concentrations of Pb and the potential for contamination limits the interpretation of the reported observations and consideration of the observed effects in the absence of analytical verification. In these cases, less weight is placed on study findings in drawing conclusions regarding the effects of Pb exposure.

In the following sections, the literature on aquatic ecosystem effects of Pb, published since the 2006 Pb AQCD, is considered with brief summaries from the 1977 Pb AQCD, the 1986 Pb AQCD and the 2006 Pb AQCD where relevant. Biogeochemistry of Pb in aquatic systems is reviewed in Section 6.4.2. Sections 6.4.3 and 6.4.4 consider the bioavailability and uptake of Pb by freshwater plants, invertebrates, and vertebrates. Biological effects of Pb on freshwater ecosystem components (plants, invertebrates, and vertebrates) are discussed in Section 6.4.5. In this section, effects are generally presented from sub-organismal responses (i.e., enzymatic activities, changes in blood parameters) to endpoints relevant to the population-level and higher (growth, reproduction and survival; summarized in Table 6-5). Biological effects are followed by data on exposure and response of freshwater species (Section 6.4.6). Effects of Pb at the freshwater ecosystem level of biological organization are discussed in Section 6.4.7. Section 6.4 includes a discussion of critical loads in freshwater systems (Section 6.4.8), characterization of sensitivity and vulnerability of freshwater ecosystem components (Section 6.4.9) and a discussion of Pb effects on ecosystem services (Section 6.4.10). A synthesis of the new evidence for Pb effects on freshwater organisms (Section 6.4.11) is followed by causal determinations based on evidence dating back to the 1977 Pb AQCD (Section 6.4.12). Corresponding sections on saltwater systems introduced in Section 6.4.13 include bioavailability of Pb in saltwater (Section 6.4.14), biological effects of Pb in saltwater (Section 6.4.15), exposure and response of saltwater species (Section 6.4.16), community and ecosystem level effects (Section 6.4.17) and characterization of sensitivity and vulnerability in saltwater species (Section 6.4.18) and ecosystem services (Section 6.4.19). The saltwater ecosystem section concludes with a synthesis of new evidence for Pb effects in marine/estuarine systems (Section 6.4.20) and

causal determinations based on evidence dating back to earlier AQCDs when available (Section 6.4.21).

6.4.2 Biogeochemistry and Chemical Effects of Pb in Freshwater and Saltwater Systems

Quantifying Pb speciation in aquatic environments is critical for determining the toxicity of the metal to aquatic organisms. As reviewed in the 2006 Pb AQCD (U.S. EPA, 2006b) and discussed in detail in Sections 2.3 and 6.2 of this assessment (Fate and Transport), the speciation process is controlled by many environmental factors. Although aerially deposited Pb largely consists of the labile Pb fraction, once the atmospherically-derived Pb enters surface waters its fate and bioavailability are influenced by Ca^{2+} concentration, pH, alkalinity, temperature, total suspended solids, and dissolved organic carbon (DOC), including humic acids. In sediments, Pb is further influenced by the presence of sulfides and Fe and Mn oxides. For instance, in neutral to acidic aquatic environments, Pb is typically present as $PbSO_4$, $PbCl_4$, Pb^{2+}, cationic forms of Pb hydroxide, and ordinary hydroxide [$Pb(OH)_2$]. In near neutral pH the solubility of Pb is low and Pb may be present as $Pb(OH)_2$, cerussite ($PbCO_3$), or hydrocerussite ($Pb_3(OH)_2(CO_3)$), while in alkaline waters, common forms of Pb include Pb carbonates [$Pb(CO_3)$] and hydroxides [$Pb(OH)_2$]. In addition to these inorganic forms, Pb humate is present in the solid phase and Pb fulvate is present in solution. In freshwater systems, Pb complexes with inorganic OH^- and CO_3^{2-} and forms weak complexes with Cl^-; conversely, Pb speciation in seawater is a function of chloride concentration and the primary species are $PbCl_3$, $PbCO_3$, $PbCl_2$, and $PbCl^+$.

In many, but not all aquatic organisms, Pb dissolved in water can be the primary exposure route to gills or other biotic ligands. The toxicity associated with Pb in the water column or sediment pore waters is directly affected by the competitive binding of Pb to the anions listed above. In aqueous exposures, Pb is typically added as either the chloride or the nitrate salt. Both dissociate completely to release lead ions, Pb^{2+}. The Pb ions can react with ligands in the solution to form soluble complexes and insoluble precipitates. For solutions of a near neutral pH and lower the solubility of Pb is low. Recent research has suggested that due to the low solubility of Pb in water, dietary Pb (i.e., lead adsorbed to sediment, particulate matter, and food) may contribute substantially to exposure and toxicity in aquatic biota (Sections 6.4.4.2 and 6.4.4.3). Factors determining solubility of Pb such as distribution of absorbed, dissolved, and solid Pb species can be estimated with aquatic speciation models discussed below.

Currently, national and state ambient water quality criteria for Pb attempt to adjust measured concentrations to better represent the bioavailable free ions, and express the criteria value as a function of the hardness (i.e., amount of Ca^{2+} and Mg ions) of the water in a specific aquatic system. Models such as the BLM (Figure 6-3) (Paquin et al., 2002; Di Toro et al., 2001) include an aquatic speciation model (WHAM V; see below) combined with a model of competitive binding to gill surfaces, and provides a more comprehensive method for expressing Pb concentrations at specific locations in terms of the bioavailable metal. Sediment quality criteria have not been established, although the EPA has developed methods based on equilibrium partitioning theory to estimate sediment benchmarks for Pb and a few other metals (U.S. EPA, 2005d). The approach is based on the ratio of the sum of simultaneously extracted metals and amount of AVS, adjusted for the fraction of organic carbon present in the sediments, and is reviewed in detail in the 2006 Pb AQCD (U.S. EPA, 2006c). It is important to note that this method cannot accurately predict which sediments are toxic or which metal is the primary risk driver.

A more detailed understanding of the biogeochemistry of Pb in aquatic systems (both the water column and sediments) is critical to accurately predicting toxic effects of Pb to aquatic organisms. It should be recognized, however, that in addition to exposure via sediment and water, chronic exposures to Pb also include dietary uptake, even though the toxicokinetics of this exposure pathway are not yet well understood in aquatic organisms and the influence of the bioavailability factors described above is unknown. Furthermore, changes in environmental factors that reduce the bioaccessible Pb fraction can result in either sequestration in sediments or subsequent release as mobile, bioaccessible forms. This section provides updated information about the influence of chemical parameters that affect Pb bioaccessibility in the aquatic environment (in sediments and the water column).

Several models are available for estimating the speciation of dissolved Pb. These models were tested by Balistrieri and Blank (2008) by comparing the speciation of dissolved Pb in aquatic systems affected by historical mining activities with that predicted by several models, including Windermere humic aqueous model (WHAM VI), non-ideal competitive absorption Donnan-type model (NICA-Donnan), and Stockholm humic model (SHM). Accurate prediction of labile Pb concentrations was achieved only with SHM, although other metal concentrations were better described by the WHAM model. Whereas both WHAM VI and NICA-Donnan predicted that the bulk of Pb contamination would be complexed with Fe, SHM predicted Pb speciation predominantly characterized by Fe and inorganic Pb complexes. Predicted dynamic Pb concentrations developed with the WHAM VI and NICA-Donnan methods overestimated Pb concentrations measured using diffusive gradients in thin-films in Lake Greifen (Switzerland), but underestimated

concentrations in Furbach stream (located in both the Coeur d'Alene and Spokane River Basins in Idaho), indicating that such models may not be able to accurately describe metal speciation under all environmental conditions (Balistrieri and Blank, 2008). Another model that can be used to calculate the equilibrium composition of dilute aqueous solutions in the laboratory or natural aqueous systems is MINTEQA2 which considers dissolved species, absorbed species, and multiple solid phases under a variety of conditions (U.S. EPA, 2006e).

Quantification of different sediment metal-binding phases, including sulfide, organic carbon (OC), Fe, and Mn phases, is important to fully understand the bioaccessible fraction of Pb and the toxicity to benthic organisms (Simpson and Batley, 2007). However, physical disturbance, pH change, and even the biota themselves also alter sediment binding or release of Pb. Atkinson et al. (2007) studied the effects of pH on sequestration or release of Pb from sediments. Although high and circumneutral water pH (8.1 and 7.2) did not affect the release of sequestered Pb from sediments, lowering the pH to 6 increased the concentration of Pb in overlying waters from less than 100 µg Pb/L to 200-300 µg Pb/L. Physical sediment disturbance also increased the amount of sediment-bound Pb released into the aqueous phase. When Pb-contaminated sediment was physically disturbed, the dissolved oxygen content of the overlying water was observed to significantly impact Pb mobilization, with greater Pb mobilization at lower dissolved oxygen levels (3 to 9 mg/L O_2) (Atkinson et al., 2007). In addition, although Pb concentrations in the sediments of a mine-impacted wetland in Hezhang, China, were determined to be strongly associated with organic/sulfide and residual fractions (e.g., 34 to 82% of total Pb), the presence of aquatic macrophytes altered the Pb speciation, increasing the fraction of Pb bound to Fe-Mn oxides (42% to 47% of total Pb) (Bi et al., 2007). This phenomenon was investigated in greater depth by Sundby et al. (2005), who determined that release of oxygen from macrophyte roots resulted in the oxidation of sediment-bound Pb, leading to the release of bioaccessible Pb fractions (Sundby et al., 2005).

6.4.2.1 Other Metals

Multiple metals are present simultaneously in many aquatic environments and may interact with one another influencing Pb uptake and toxicity. Interactions of Pb with other metals were reviewed in the 2006 Pb AQCD, and more recent evidence supports previous findings of altered bioavailability associated with metal mixtures. Komjarova and Blust (2008) looked at the effect of the presence of Cd^{2+} on the uptake of Pb by the freshwater cladoceran *Daphnia magna*. While Pb uptake rates were not affected by Cu, Ni or Zn, enhanced Pb accumulation was observed in the presence of 0.2 µM Cd. The highest Pb

concentration, 0.25 µM (51.8 µg Pb/L) in turn facilitated Cu uptake. Area-specific and whole organism Pb transport rates were greatest in the mid-intestine. It was concluded that Pb-induced disruptions of ion homeostasis and metal absorption processes might be a possible explanation of stimulated Pb uptake in the presence of Cd, as well as the increase in Cu uptake rates provoked by presence of Pb at its highest studied concentration. Komjarova and Blust (2009b) then considered the effect of Na, Ca^{2+} and pH on simultaneous uptake of Cd, Cu, Ni, Pb and Zn. Cd and Pb showed increased uptake rates at high Na concentration. It was thought that increased Na uptake rates promoted Pb entrance to the cell. With respect to the effect of pH, reduced proton competition begins to influence Pb uptake in waters with high pH. A clear suppression of Cd, Ni, Pb and Zn uptake was observed in the presence of Ca^{2+} (2.5 mM). Ca^{2+} has been reported to have a protective effect in other studies (involving other organisms). The presence of other metals may also affect the uptake of Pb by fish. At low concentrations, Cd in a Pb-Cd mixture out-competed Pb at gill tissue binding sites in rainbow trout (*Oncorhynchus mykiss*), resulting in a less-than additive toxicity when fish were exposed to both metals in tandem (Birceanu et al., 2008). Evidence for the presence of Pb influencing the uptake of other metals was observed in the marine bivalves *Macomona liliana* and *Austrovenus stutchburyi*. Significantly, more Zn bioaccumulated in the presence of Pb in these mussels than with Zn alone following a 10-day exposure to spiked sediments (Fukunaga and Anderson, 2011).

6.4.2.2 Biofilm

Farag et al. (2007) measured Pb concentrations in various media (water, colloids, sediment, biofilm) as well as invertebrates and fish collected within the Boulder River watershed, MT, U.S. They concluded that the fraction of Pb associated with Fe-oxides was most frequently transferred to biofilms and the other biological components of the sampled systems (Farag et al., 2007). Consequently, an increase in the Pb Fe-oxide fraction could signify a potential increase in the bioaccessible pool of Pb. The authors also noted that this fraction may promote downstream transport of Pb contamination. Ancion et al. (2010) investigated whether urban runoff metal contaminants could modify biofilm bacterial community structure and diversity and therefore potentially alter the function of biofilms in stream ecosystems. They found that accumulation rates for metals in biofilm were maximal during the first day of exposure and then decreased with time. Equilibrium between metal concentrations in the water and in the biofilm was reached for all metals after 7-14 days of exposure. The affinity of the biofilm for Pb was, however, much greater than for Cu and Zn. With respect to recovery, the release of metals was slow and after 14 days in clean water 35% of Pb remained in the biofilm. By retaining

and releasing such metal pollutants, biofilms may play a key role in determining both the concentration of the dissolved metals in the water column and the transfer of the metals to invertebrates and fish grazing on them. An enrichment factor of 6,000:1 for Pb between the biofilm and the water was measured after 21 days exposure to synthetic urban runoff. The relatively slow release of such metal may greatly influence the transfer of Pb to organisms feeding on the biofilms. This may be of particular importance during storm events when large amounts of Pb are present in the urban runoff. It was suggested that biofilms constitute an integrative indicator of metal exposure over a period of days to weeks.

6.4.2.3 Carbonate

An investigation of heavy metal concentrations in an industrially impacted French canal (Deule canal) indicated that total extractable Pb in sediments ranged from 27 to 10,079 mg Pb/kg, with 52.3% present in Fe-Mn oxide fractions, 26.9% as organic sulfide fraction, 10.7% in carbonates, and 10.1% in the residual fraction (Boughriet et al., 2007). The relatively high fraction of Pb associated with carbonates was not observed at other sites, as sediments in these areas contained low proportions of carbonates. Hence, addition of carbonates (either from anthropogenic or natural sources) can significantly impact Pb speciation in sediments, and potential bioavailability to resident organisms. In addition, increased surface water carbonate concentrations also reduced the bioaccessible Pb fraction as measured by chronic Pb accumulation in the fathead minnow, (*Pimephales promelas*) (Mager et al., 2010), and by Pb toxicity to fathead minnow and the cladoceran (*Ceriodaphnia dubia*) (Mager et al., 2011b).

6.4.2.4 Dissolved Organic Matter (DOM)

Uptake of Pb by water-column organisms is affected by the concentration of DOM (Mager et al., 2011a; Mager et al., 2010). In a 7-day chronic study with *C. dubia*, DOM protected against toxicity while water hardness was not protective (Mager et al., 2011a). The specific composition of DOM has been shown to affect the bioaccessibility of environmental Pb. Humic acid-rich DOM resulted in decreased free Pb ion concentration when compared to systems containing DOM with high concentrations of polysaccharides (Lamelas and Slaveykova, 2008). When the sequestering abilities of various components of DOM were compared, humic acid again was shown to be most efficient at reducing the Pb free ion concentration, followed by fulvic acid, alginic acid, polygalacturonic acid, succinoglycan, and xanthan (Lamelas et al., 2005). Lamelas et al. (2009) considered the effect of humic acid on Pb(II) uptake by freshwater algae taking account of kinetics and

cell wall speciation. The uptake flux was described by a Michaelis-Menten type equation. Comparison of Cu(II), Cd(II) and Pb(II) uptake by green freshwater algae, (*Chlorella kessleri),* in the presence of either citric acid or humic acid was made. The uptake fluxes, percentage adsorbed and percentage internalized for Cu and Cd were identical in the presence of either citric or humic acid. In contrast, however, there was a ten-fold increase in the respective values for Pb. The increase in adsorbed Pb was attributed to the increase in adsorption sites from the adsorbed humic acid on the surface of the algae. Two hypotheses were considered to explain the increase in internalized Pb and the internalization flux: (1) direct interaction of Pb-humic acid complexes with the internalization sites, and (2) uptake of Pb(II) after dissociation from the Pb-humic acid complex. The authors favor the former hypothesis but no evidence is presented for the proposed ternary Pb-humic acid-internalized site complexes, nor is there an explanation as to why this behavior is not observed for Cd or Cu.

There is evidence, however, that DOC/DOM does not have the same effect on free Pb ion concentration in marine systems as in freshwater systems. No correlation was observed between DOM concentration or composition and Pb toxicity when examined using the sea urchin (*Paracentrotus lividus)* embryo-larval bioassay (Sanchez-Marin et al., 2010a). For marine invertebrates, the presence of humic acid increased both the uptake and toxicity of Pb, despite the fact that a larger fraction of Pb is complexed with humic acid (25 to 75%). Although the authors could not provide a precise explanation for this, they theorized that in marine environments, addition of humic acid could induce and enhance uptake of Pb via membrane Ca^{2+} channels (Sanchez-Marin et al., 2010b). This mechanism was observed in the marine diatom (*Thalassiosira weissflogii),* in that humic acids absorbed to cell surfaces increased metal uptake; however, water column Pb-humic acid associations did appear to reduce free Pb ion concentrations (Sanchez-Marin et al., 2010b). Formation of a ternary complex that is better absorbed by biological membranes was another proposed mechanism that could describe the increased bioaccessibility to marine invertebrates of Pb bound to humic acid (Sánchez-Marín et al., 2007).

Sanchez-Marin and Beiras (2011) subsequently have shown that different components of DOM have different effects on Pb bioavailability in marine systems. Their initial research using Aldrich humic acid found that increasing humic acid concentrations increased Pb uptake by mussel gills and increased toxicity to sea urchin larvae in marine environments (Sánchez-Marín et al., 2007). In contrast, a subsequent investigation found that fulvic acid reduced Pb bioavailability in marine water (Sánchez-Marín et al., 2011). The contradictory effects of different components of DOM on marine bioavailability likely reflect their distinct physico-chemical characteristics. More hydrophobic than fulvic acid, humic acid may adsorb directly to cell membranes and enhance Pb uptake through some (still unidentified) mechanism (Sánchez-Marín et al., 2011).

Humic acid may increase the bioaccessible Pb fraction for green algae through formation of a ternary complex that promotes algal uptake of the metal. Lamelas and Slaveykova (2007) found that aqueous Pb formed complexes with humic acid, which in turn would become adsorbed to *C. kesslerii* algal surfaces, and that the presence of Pb sorbed to humic acid did not interfere with humic acid-algae complexation. The authors concluded that humic acids bound to algae acted as additional binding sites for Pb, thus increasing the concentrations associated with the algal fraction (Lamelas and Slaveykova, 2007).

Based on the above, the recent literature indicates the existence of a number of deviations from current models used to predict bioaccessibility of Pb. In marine aquatic systems, for instance, surface water DOM was found to increase (rather than decrease) uptake of Pb by fish gill structures, potentially through the alteration of membrane Ca^{2+}-channel permeability. This phenomenon would not be accurately predicted by a BLM developed using data from freshwater organisms. Further, in both freshwater and marine environments, algal biosorption of labile Pb fraction was also increased by humic acid and DOM, likely through the formation of ternary complexes that increase Pb binding sites on the algal surface. Although it is unclear whether Pb in this form is available for toxic action on algae, it is likely to comprise a significant source of dietary Pb for primary consumers. Moreover, in an attempted field verification of freshwater bioaccessibility models conducted at sites upstream, within and downstream of a mining district only one model (SHM) adequately predicted Pb bioaccessibility (Balistrieri and Blank, 2008).

6.4.2.5 Sulfides

In sediments, Pb bioavailability is further influenced by sulfides. In the presence of sulfides, most of the reactive metal in sediments will form insoluble metal sulfide that is not bioavailable for uptake by benthic organisms. Acid volatile sulfide (AVS) has been used to predict the toxicity of Pb and other metals in sediments (Ankley et al., 1996; Di Toro et al., 1992) and in the development of sediment quality criteria (Section 6.4.3). The role of sulfides in the flux of Pb from sediments is discussed further in Section 2.3.2.3.

6.4.3 Introduction to Bioavailability and Biological Effects of Pb in Freshwater Ecosystems

Freshwater ecosystems across the U.S. encompass many habitats including ponds, streams, rivers, wetlands and lakes. Concentrations of Pb available for fresh surface-water and freshwater sediments are reported in Section 6.2.3 and Table 6-2 and are

summarized here. Representative median and range of Pb concentrations in surface waters (median 0.50 µg Pb/L, range 0.04 to 30 µg Pb/L), sediments (median 28 mg Pb/kg dry weight, range 0.5 to 12,000 mg Pb/kg dry weight) and fish tissues (geometric mean 0.54 mg Pb/kg dry weight, range 0.08 to 23 mg Pb/kg dry weight [whole body]) in the U.S. based on a synthesis of NAWQA data reported in the previous 2006 Pb AQCD (U.S. EPA, 2006c). Additional information on ambient Pb levels in waters, sediments and biota is presented in Section 2.6.5 and Table 6-2 including new data from the Western Airborne Contaminants Assessment Project (WACAP) on Pb in environmental media and biota from remote ecosystems in the western U.S. WACAP assessed concentrations of semi-volatile organic compounds and metals in up to seven ecosystem components (air, snow, water, sediment, lichen, conifer needles and fish) in watersheds of eight core national parks during a multi-year project conducted from 2002-2007 (Landers et al., 2008). The goals of the study were to assess where these contaminants were accumulating in remote ecosystems in the western U.S., identify ecological receptors for the pollutants, and to determine the source of the air masses most likely to have transported the contaminants to the parks.

The 2006 Pb AQCD (U.S. EPA, 2006b) provided an overview of regulatory considerations for water and sediments in addition to consideration of biological effects and major environmental factors that modify the response of aquatic organisms to Pb exposure. Regulatory guidelines for Pb in water and sediments have not changed since the 2006 Pb AQCD, and are summarized below with consideration of limited new information on these criteria since the last review. This section is followed by new information on biogeochemistry, bioavailability and biological effects of Pb since the 2006 Pb AQCD.

The most recent ambient water quality criteria for Pb in freshwater were released in 1985 (U.S. EPA, 1985) by the EPA Office of Water which employed empirical regressions between observed toxicity and water hardness to develop hardness-dependent equations for acute and chronic criterion. These criteria are published pursuant to Section 304(a) of the Clean Water Act and provide guidance to states and tribes to use in adopting water quality standards for the protection of aquatic life and human health in surface water. The ambient water quality criteria for Pb are expressed as a criteria maximum concentration (CMC) for acute toxicity and criterion continuous concentration (CCC) for chronic toxicity (U.S. EPA, 2009b). In freshwater, the CMC is 65 µg Pb/L and the CCC is 2.5 µg Pb/L at a hardness of 100 mg/L.

The 2006 Pb AQCD summarized two approaches for establishing sediment criteria for Pb based on either bulk sediment or equilibrium partitioning (Section 6.2.1 and Section AX7.2.1.4). The first approach is based on empirical correlations between metal

concentrations in bulk sediment and associated biological effects to derive threshold effect concentrations (TEC) and probable effects concentrations (PEC) (MacDonald et al., 2000). The TEC/PEC approach derives numeric guidelines to compare against bulk sediment concentrations of Pb. The other approach in the 2006 Pb AQCD was the equilibrium partitioning procedure published by the EPA for developing sediment criteria for metals (U.S. EPA, 2005d). The equilibrium partitioning approach considers bioavailability by relating sediment toxicity to pore water concentration of metals. The amount of simultaneously extracted metal (SEM) is compared with the metals extracted via AVS since metals that bind to AVS (such as Pb) should not be toxic in sediments where AVS occurs in greater quantities than SEM.

Since the 2006 Pb AQCD, both of these methods for estimating sediment criteria for metals, have continued to be used and refined. The SEM approach was further refined in the development of the sediment BLM (Di Toro et al., 2005). The BLM is discussed further in Sections 6.3.3 and 6.4.4 . Comparison of empirical approaches with AVS-SEM in metal contaminated field sediments shows that samples where either method predicted there should be no toxicity due to metals, no toxicity was observed in chronic amphipod exposures (Besser et al., 2009; MacDonald et al., 2009). However, when the relationship between invertebrate habitat (epibenthic and benthic) and environmental Pb bioaccumulation was investigated, De Jonge et al. (2010) determined that different environmental fractions of Pb were responsible for invertebrate uptake and exposure. Pb uptake by benthic invertebrate taxa was not significantly correlated to AVS Pb levels, but rather to total sediment concentrations (De Jonge et al., 2009). Conversely, epibenthic invertebrate Pb body burdens were better correlated to AVS concentrations, rather than total Pb sediment concentrations (De Jonge et al., 2010).

In the following sections, recent information since the 2006 Pb AQCD on Pb in freshwater ecosystems will be presented. Throughout the sections, brief summaries of conclusions from the 1977 Pb AQCD, the 1986 Pb AQCD and 2006 Pb AQCD are included where appropriate. The sections are organized to consider uptake of Pb and effects at the species level, followed by community and ecosystem level effects. New research on the bioavailability and uptake of Pb into freshwater organisms including plants, invertebrates, and vertebrates is presented in Section 6.4.4. Effects of Pb on the physiology of freshwater flora and fauna (Section 6.4.5) are followed with data on exposure and response of freshwater organisms (Section 6.4.6). Responses at the community and ecosystem levels of biological organization are reviewed in Section 6.4.7 followed by a brief consideration of critical loads in freshwater systems (Section 6.4.8), characterization of sensitivity and vulnerability of ecosystem components (Section 6.4.9) and a discussion of ecosystem services (Section 6.4.10). The freshwater ecosystem

section concludes with a synthesis of new evidence (Section 6.4.11) and causal determinations based on evidence dating back to the 1977 Pb AQCD (Section 6.4.12).

6.4.4 Bioavailability in Freshwater Systems

Bioavailability was defined in the 2006 Pb AQCD as "the proportion of a toxin that passes a physiological membrane (the plasma membrane in plants or the gut wall in animals) and reaches a target receptor (cytosol or blood)." In 2007, EPA took cases of bioactive adsorption into consideration and revised the definition of bioavailability as "the extent to which bioaccessible metals absorb onto, or into, and across biological membranes of organisms, expressed as a fraction of the total amount of metal the organism is proximately exposed to (at the sorption surface) during a given time and under defined conditions" (U.S. EPA, 2007c). See Section 6.3.3 for additional discussion of bioavailability.

The bioavailability of metals varies widely depending on the physical, chemical, and biological conditions under which an organism is exposed (U.S. EPA, 2007c). The bioavailability of a metal is also dependent upon the bioaccessible fraction of metal. The bioaccessible fraction of a metal is the portion (fraction or percentage) of environmentally available metal that actually interacts at the organism's contact surface and is potentially available for absorption or adsorption by the organism (U.S. EPA, 2007c). The processes for evaluating bioavailability and bioaccessibility are presented in Figure 6-2 and in Section 6.3.3. In brief, trace metals, and their complexes, must first diffuse from the external medium to the surface of the organism (mass transport). Metal complexes may dissociate and re-associate in the time that it takes to diffuse to the biological surface. These processes are considered further in Chapter 2. To have an effect on the organism, metals must then react with a sensitive site on the biological membrane (adsorption/desorption processes), often but not necessarily followed by biological transport (internalization). Any of these processes may be the rate limiting step for the overall biouptake process. Internalization is, however, the key step in the overall biouptake process. Although the transport sites often have a high affinity for required metals they do not always have high selectivity and so a toxic metal may bind to the site of an essential metal with a similar ionic radius or co-ordination geometry, e.g., Pb^{2+}, Cd^{2+} and Zn^{2+} are similar to Ca^{2+}. At the molecular level, there are three major classes of transition metal transporter: P-type ATPases, Zn regulated transporter/Fe-regulated transporter, and natural resistance associated macrophage proteins (Worms et al., 2006). Of these, natural resistance associated macrophage proteins have been shown to promote the uptake of various metals including Pb. This type of trace metal transport can be described by Michaelis-Menten uptake kinetics and equilibrium considerations.

Routes of Exposure

According to the 2006 Pb AQCD (U.S. EPA, 2006b), Pb adsorption, complexation, chelation, etc., are processes that alter its bioavailability to different aquatic species, and it was suggested that multiple exposure routes may be important in determining overall bioavailability of Pb. Given its low solubility in water, bioaccumulation of Pb by aquatic organisms may preferentially occur via exposure routes other than direct absorption from the water column, including ingestion of contaminated food and water, uptake from sediment pore waters, or incidental ingestion of sediment. If uptake and accumulation are sufficiently faster than depuration and excretion, Pb tissue levels may become sufficiently high to result in physiological effects (Luoma and Rainbow, 2005). Pb accumulation rates are controlled, in part, by metabolic rate. Other factors that influence bioavailability of Pb to organisms in aquatic systems are reviewed in Section 6.4.2. As summarized in the 2006 Pb AQCD, organisms exhibit three Pb accumulation strategies: (1) accumulation of significant Pb concentrations with low rate of loss resulting in substantial accumulation; (2) balance between excretion and bioavailable metal in the environment; and (3) very low metal uptake rate without significant excretion, resulting in weak net accumulation (Rainbow, 1996). Uptake experiments with aquatic plants, invertebrates, and vertebrates reviewed in the 2006 Pb AQCD showed increases in Pb uptake with increasing Pb in solution. The 2006 Pb AQCD findings included consideration of bioaccumulation in different trophic levels. Pb concentrations were found to be typically higher in algae and benthic organisms and lower in higher trophic-level consumers.

In this section:

1) Recent information on bioavailability and uptake in algae, plants, invertebrates and vertebrates from freshwater systems are reviewed with summary material from the 2006 Pb AQCD and earlier Pb AQCDs where appropriate.
2) An overview of the BLM is presented as the most widely used method for predicting both the bioaccessible and bioavailable fractions of Pb in the aquatic environment.
3) Bioavailability in algae, plants, invertebrates and vertebrates is discussed. As reviewed by Wang and Rainbow (2008), aquatic organisms exhibit distinct patterns of metal bioaccumulation. The authors suggest that the observed differences in accumulation, body burden, and elimination between species are due to metal biogeochemistry and physiological and biological responses of the organism. The studies presented below generally support the observations of Wang and Rainbow (2008) that closely related species can vary greatly in bioaccumulation of Pb and other non-essential metals.

The bioaccumulation and toxicity of Pb to aquatic organisms are closely linked to the environmental fate of the metal under variable environmental conditions (Section 2.3) as they are highly dependent upon the relative proportion of free metal ions in the water column. However, information is lacking on the uptake of Pb through ingestion of Pb-sorbed particles or dietary exposure to biologically-incorporated Pb. Such routes of exposure are not included in models such as the BLM that predict toxicity as a function of Pb concentration in the water column. This uncertainty may be greater for Pb than for other more soluble metals (such as Cu) as a greater proportion of the total mass of Pb in an aquatic ecosystem is likely to be bound to particulate matter. Therefore, estimating chronic toxicity of Pb to aquatic receptors may have greater uncertainty than predicting acute effects.

BLM Models

In addition to the biogeochemical effects that govern the environmental pool of accessible Pb, reactions of Pb with biological surfaces and membranes determines the bioavailability and uptake of the metal by aquatic organisms. The BLM (Figure 6-3) predicts both the bioaccessible and bioavailable fraction of Pb in the aquatic environment, and can be used to estimate the importance of environmental variables such as DOC in limiting uptake by aquatic organisms (Alonso-Castro et al., 2009). The BLM integrates the binding affinities of various natural ligands in surface waters and the biological uptake rates of aquatic organisms to determine the site-specific toxicity of the bioavailable fraction.

In the 2006 Pb AQCD, limitations of the use of BLM in developing air quality criteria were recognized including the focus of this model on acute endpoints and the absence of consideration of dietary uptake as a route of exposure. Atmospheric deposition of Pb to aquatic systems and subsequent effects on ecosystem receptors is likely characterized as a chronic, cumulative exposure rather than an acute exposure. Recommendations from the 2006 Pb AQCD included developing both chronic toxicity BLMs and BLMs that consider the dietary route of Pb uptake. The EPA recently incorporated the BLM into the Framework for Metal Risk Assessment (U.S. EPA, 2007c) and has published an ambient freshwater criteria document for Cu based on the BLM model (U.S. EPA, 2007a). This section reviews the literature from the past 5 years on applications of the BLM to predicting bioavailability of Pb to aquatic organisms. However, the primary focus of initial BLMs has been acute toxicity endpoints for fish and invertebrates following gill or cuticular uptake of metals.

Di Toro et al. (2005) constructed BLMs for metals exposure in sediments, surface water, and sediment pore water to determine how to most accurately predict the toxicity of metals-contaminated sediments. Results from models were compared with literature-

derived acute toxicity values for benthic and epibenthic invertebrates to establish the accuracy of the developed models. Although the models tended to overestimate the toxicity of aqueous and sediment-bound Pb in freshwater environments, it was determined that the model significantly underestimated Pb toxicity to marine invertebrates (Di Toro et al., 2005). This may be because pore water metal concentrations were not modeled. Consequently, these results may suggest that either 1) mobilization of Pb concentrations from sediments into pore water is greater in marine environments, or 2) marine invertebrates are significantly more sensitive to Pb exposures than are freshwater species.

A number of deviations from results predicted by Pb exposure models (such as the BLM) were documented by Ahlf et al. (2009). They highlighted that uptake of metals by sediment-dwelling bivalves was significantly greater than predicted, because bivalves accumulate Pb from multiple sources not included in the model, such as ingestion of algae, bacteria, and colloidal matter. Species-specific dietary assimilation of ingested particulate-bound metals is also likely to play a role in the toxicity of Pb to aquatic organisms, yet insufficient data are available to permit modeling of this additional factor (Ahlf et al., 2009). The authors outlined the need for additional data in developing bioavailability models for chronic metal exposures. As recent evidence suggests that the hydrophobic DOC fraction (e.g., humic and fulvic acids) sequesters the greatest fraction of Pb in aquatic systems (Pernet-Coudrier et al., 2011), understanding the influence of this adsorption on Pb toxicity is critical for the prediction of chronic aquatic Pb toxicity. For instance, although the presence of humic acid is considered to reduce the bioavailable fraction of metals in surface water, green algae uptake and biosorption of metals, including Pb, was actually increased by humic acid. The authors determined that humic acid bound to algal surfaces served to increase the total number of metal binding sites over those afforded solely by the algal surface (Lamelas and Slaveykova, 2007). This highlights the complexity of modeling chronic metals bioavailability through multiple exposure routes, as humic acid would decrease gill or cuticular uptake of metals from the water column, but could potentially enhance dietary exposure by increasing algal metal content. Slaveykova and Wilkinson (2005) also noted that humic acid is likely to interact with other biological membranes and alter their permeability to metals, especially in acidic environments. Further, they observed that increased surface water temperatures not only increase membrane permeability but also change metabolic rates, both of which can enhance metals uptake and assimilation; however, this factor is not included in bioavailability models such as the BLM (Slaveykova and Wilkinson, 2005). Despite this, the authors noted that, in most cases, the BLM could predict acute metals toxicity with a reasonable degree of accuracy.

6.4.4.1 Freshwater Plants and Algae

In the 1977 Pb AQCD, the root system of plants was recognized as the major route of uptake for Pb (U.S. EPA, 1977). Uptake and translocation studies of Pb in plants and algae reviewed in the 1977 Pb AQCD and the 2006 Pb AQCD indicated that plants tend to sequester larger amounts of Pb in their roots than in their shoots. Recent studies on bioavailability of Pb to plants support the findings of the previous Pb AQCDs and provide additional evidence for species-dependent differences in responses to Pb in water and sediments.

Most biouptake studies in aquatic plants and algae available since the 2006 Pb AQCD, were typically conducted at very high concentrations of Pb that are not representative of current levels of Pb typically encountered in freshwater. However, most of these exposures included a series of increasing concentrations of Pb and generally, Pb was accumulated in a dose-dependent manner. Studies in which high concentrations of Pb are used and an exposure-response relationship is observed may imply effects at lower concentrations but uncertainty remains to the extent to which effects would be observed at concentrations of Pb typically found in the environment. The role of modifying factors, such as the presence of other metals, on uptake rates as well as species differences in Pb uptake rates can be determined from experimental Pb concentrations that are higher than measured Pb in the environment. Plants that are hyperaccumulators of Pb and other metals may be used for phytoremediation at highly contaminated sites and there is a large body of literature on uptake of very high concentrations of metals by different species. This chapter focuses on environmentally relevant concentrations of Pb and also those studies with doses or exposures in the range of one or two orders of magnitude above current or ambient conditions, as described in the Preamble. In freshwater ecosystems in the U.S., the average Pb concentration in surface water is 0.5 µg Pb/L (Table 6-2), however, total Pb in water has been measured as high as 2,000 µg Pb/L where mining operations have affected streams (Table 2-11).Studies with freshwater algae available since the 2006 Pb AQCD, are primarily limited to nominal media exposures at high concentrations of Pb with metal quantified in tissues. For example, the microalgae *Spirulina platensis* was demonstrated to accumulate Pb from Zarrouk culture medium in a concentration-dependent manner with nominal initial concentrations of 5,000, 10,000, 30,000, 50,000 and 100,000 µg Pb/L (Pb in medium was measured every two days thereafter), following a 10-day incubation period (Arunakumara et al., 2008). Pb concentrations accumulated by algae appeared to decrease when culture time increased from 2 to 10 days. This may have occurred as a result of a gradual recovery of growth and an addition of biomass that would have reduced the concentration of Pb in algal tissue. An aquatic moss, *Fontinalis antipyretica*, accumulated up to an average of 622 mg Pb/kg dry weight over a 7-day nominal exposure to 20,700 µg Pb/L (Rau et al., 2007).

Interestingly, experimentation with concurrent Cu and Pb exposure indicated that the presence of Cu increased the uptake of Pb by the green algae *Chlamydomonas reinhardtii* (Chen et al., 2010c). The authors noted that, in the case of Cu-Pb binary exposures, uptake rates of Pb exhibited complex non-linear dynamics in other aquatic organisms as well.

Additional uptake studies conducted since the 2006 Pb AQCD include new information for freshwater macrophytes. When exposed to nominal water concentrations of up to 20,700 µg Pb/L, floating (non-rooted) coontail plants (*Ceratophyllum demersum*) accumulated an average Pb concentration of 1,748 mg Pb/kg after 7 days, although this was not significantly higher than levels accumulated in the first day of exposure (Mishra et al., 2006b). Induction of the antioxidant system improved the tolerance of the aquatic plant *Najas indica* for bioaccumulated Pb, allowing for increased biomass and the potential to accumulate additional Pb mass. High Pb accumulation (3,554 mg Pb/kg dry weight tissue following a 7-day exposure to 20,720 µg Pb/L) was considered to be a function of plant morphology; as a submerged, floating plant, *N. indica* provides a large surface area for the absorption of Pb (Singh et al., 2010).

Given that atmospherically-derived Pb is likely to become sequestered in sediments (Section 6.2), uptake by aquatic macrophytes is a significant route of Pb removal from sediments, and a potential route for Pb mobilization into the aquatic food web. The rooted aquatic macrophyte *Eleocharis acicularis* was determined to be a hyperaccumulator of Pb in an 11-month bioaccumulation experiment with mine tailings. When grown in sediments containing 1,930 mg Pb/kg, the maximum concentration of Pb in *E. acicularis* was determined to be 1,120 mg Pb/kg dry weight. However, calculated BCFs for Pb were all less than one, indicating that Pb uptake, although high, was less efficient than for other metals present (Ha et al., 2009).

Aquatic plants inhabiting a wetland containing an average sediment Pb concentration of 99 mg Pb/kg exhibited variable Pb tissue concentrations, but these do not appear to be related to macrophyte type (e.g., submerged, floating, emergent, etc.). Consequently, the authors concluded that uptake of Pb by aquatic plants appears to be dependent on species, at the exclusion of habitat or type. For instance, among the submerged plant species, *Ceratophyllum demersum* accumulated the greatest amount of Pb (22 mg Pb/kg dry weight), while *Potamogeton malainus* tissue contained the least amount of Pb, 2.4 mg Pb/kg dry weight (Bi et al., 2007). Tissues of the floating plants *Azolla imbricata* and *Spirogyra communis* were found to contain 12 and 20 mg Pb/kg dry weight, respectively, while emergent macrophytes *Scirpus triqueter* and *Alternanthera philoxeroides* accumulated 1.4 and 10 mg Pb/kg dry weight. Fritioff and Greger (2006) determined that anywhere from 24–59% of the total Pb taken up by *Potamogeton natans* aquatic plants

was sequestered in the cell wall fraction, depending on plant tissue and environmental Pb concentration. More importantly, no translocation of Pb was observed when plant tissues (leaf, stem, root) were exposed to Pb solutions separately (Fritioff and Greger, 2006).

Dwivedi et al. (2008) reared nine different species of aquatic plants in a fly-ash contaminated medium containing approximately 7 mg Pb/kg dry weight. Not only did species exhibit different Pb accumulation efficiencies but they also compartmentalized sequestered Pb differently. The submerged macrophyte *Hydrilla verticillata* accumulated the greatest amount of Pb (approximately 180 mg Pb/kg dry weight tissue), but Pb was sequestered solely in the shoot tissue. In contrast, other plant species accumulated between 15 and 100 mg Pb/kg dry weight (*Ranunculus scloralus* and *Marsilea quadrifolia*) with the majority compartmentalizing the metal in root tissue, except for *C. demersum* and *M. quadrifolia*, which also utilized shoot tissue for Pb storage (Dwivedi et al., 2008).

Pb concentrations in the root, leaf, and stem tissues of three aquatic plant species were found to correlate most closely with the concentration of the exchangeable Pb fraction (e.g., the fraction of Pb that is easily and freely leachable from the sediment). Authors noted that seasonal variations can alter the amount of Pb present in the exchangeable fraction, and that Pb was more likely than Cd or Cu to remain tightly bound to sediments, and therefore the relationship between total sediment Pb and Pb in aquatic plant tissues was weaker (Ebrahimpour and Mushrifah, 2009).

Lemna sp., a free floating macrophyte, incubated in a water extract of waste ash containing 19 µg Pb/L accumulated 3.5 mg Pb/kg dry weight over 7 days of exposure. Slight toxic effects, including suppression of growth, were observed over this exposure period, but this may have been a result of exposures to multiple metals in the water extract, including Cr, Mn, Cu, and Zn (Horvat et al., 2007). Lemna sp. was also demonstrated to be effective in the biosorption of Pb from solution, even in the presence of sediments (1 g per 700 mL water). Over 7 days of exposure to 3,600 and 7,000 µg Pb/L, plant biomass was found to contain an average of 2,900 and 6,600 mg/kg (wet weight) Pb, respectively, versus 200 and 300 mg/kg (dry weight) in sediment (Hurd and Sternberg, 2008).

Young *Typha latifolia*, another rooted macrophyte, were grown in analytically verified concentrations of 5,000 and 7,500 µg/L Pb-spiked sediment for 10 days to determine their value as metal accumulators. Within the exposure period, plants exposed to the lower concentration were able to remove 89% of Pb, while 84% of the Pb present in the higher treatment was taken up by *T. latifolia*. Pb concentrations measured in root and leaf tissue ranged from 1,365 to 4,867 mg Pb/kg and 272 to 927 mg Pb/kg, respectively, and were higher at the greater Pb exposure (Alonso-Castro et al., 2009).

Uptake studies available for aquatic macrophytes since the 2006 Pb AQCD include some studies where Pb was measured in field collected plants growing in metal-contaminated areas. Common reeds (*Phragmites australis*) grown in metal-impacted aquatic environments in Sicily, Italy, preferentially accumulated Pb in root and rhizome tissues (Bonanno and Lo Giudice, 2010). Pb concentrations in water and sediment averaged 0.4 µg Pb/L and 2.7 mg Pb/kg. These levels yielded root and rhizome concentrations of 17 and 15 mg Pb/kg, respectively, whereas stem and leaf Pb concentrations were lower (9.9 and 13 mg Pb/kg). These tissue concentrations were significantly correlated to both water and sediment concentrations (Bonanno and Lo Giudice, 2010). Conversely, the semi-aquatic plant *Ammania baccifera*, grown in mine tailings containing 35 to 78 mg Pb/kg, did not accumulate analytically detectable levels of Pb in either root or shoot tissues, despite the fact that other metals (Cu, Ni, Zn) were bioaccumulated (Das and Maiti, 2007). This would indicate that at low/moderate environmental Pb concentrations, some plant species may not bioaccumulate significant (or measurable) levels of Pb.

The average concentration of Pb in the tissues of rooted aquatic macrophytes (*Callitriche verna*, *P. natans*, *C. demersum*, *Polygonum amphibium*, *Veronica beccabunga*) collected from two metals-polluted streams in Poland (average sediment concentration 38 to 58 mg Pb/kg) was less than 30 mg Pb/kg. Pb bioaccumulation in plants was significantly correlated with sediment Pb concentrations (Samecka-Cymerman and Kempers, 2007). A similar significant correlation was established between reed sweet grass root Pb concentration and sediment Pb concentrations (Skorbiowicz, 2006).

Pb tissue concentrations of aquatic plants *P. australis* and *Ludwigia prostrata* collected from wetlands containing an average of 52 mg Pb/kg in surficial sediments were predominantly in root tissues, indicating poor translocation of Pb from roots. In the former, Pb decreased from an average of 37 mg Pb/kg in roots to 17, 14, and 12 mg Pb/kg in rhizome, stem and leaf tissues, respectively, while *L. prostrata* Pb tissue concentrations decreased from 77 mg Pb/kg in fibrous root to 7 and 43 mg Pb/kg in stem and leaf tissues (Yang et al., 2008a). The authors proposed that this diminished transfer ability explained the relatively low BCFs for Pb uptake in these two species, when compared with those of other metals.

Despite no significant seasonal effect on surface water Pb concentrations, shining pondweed (*Potamogeton lucens*), a rooted aquatic macrophyte grown in an urbanized metal-contaminated lake in Turkey, exhibited seasonal alterations in Pb tissue concentrations. Average water Pb concentrations were 28 µg Pb/L in spring, 27 µg Pb/L in summer, and 30 µg Pb/L in autumn. Over this same time period, root tissue Pb concentrations significantly increased from 6 mg Pb/kg dry weight in spring, to 9 mg Pb/kg dry weight in summer, and to 10 mg Pb/kg dry weight in autumn (Duman et al.,

2006). No differences were detected in stem Pb concentrations between spring and summer (approximately 4 mg Pb/kg dry weight), but stem Pb concentrations were found to be significantly higher in autumn (6 mg Pb/kg dry weight). In the same system, *P. australis* plants accumulated the most Pb during winter: 103, 23, and 21 mg Pb/kg dry weight in root, rhizome, and shoot tissue, respectively, in sediments containing 13 mg Pb/kg dry weight. By contrast, *Schoenoplectus lacustris* accumulated maximum rhizome and stem Pb concentrations of 5.1 and 7.3 mg Pb/kg dry weight in winter, but sequestered the greatest amount of Pb in root tissues during the spring (30 mg Pb/kg dry weight) at a comparable sediment concentration, 18 mg Pb/kg dry weight (Duman et al., 2007). The authors suggest that this indicated that metal uptake was regulated differently between species.

Tree species that inhabit semi-aquatic environments have also been shown to absorb Pb from Pb-contaminated sediments. Bald-cypress trees (*Taxodium distichum*) growing in sediments of a refinery-impacted bayou in Louisiana accumulated significantly greater amounts of Pb than did trees of the same species growing in bankside soil, despite the lower Pb concentrations of sediments. Bankside soils contained greater than 2,700 mg Pb/kg versus concentrations of 10 to 424 mg Pb/kg in sediments, yet Pb concentrations in trees averaged 4.5 and 7.8 mg Pb/kg tissue, respectively (Devall et al., 2006). The authors theorized that Pb was more readily released from sediments and that soil dispersion to the swamp sediments provides additional, if periodic, loads of Pb into the system. Willow seedlings planted in Pb-contaminated sediment were more effective at removing Pb from the media than a diffusive gradient in thin film technique predicted (Jakl et al., 2009). The authors proposed that the plant's active mobilization of nutrients from soil during growth also resulted in increased Pb uptake and sequestration.

Given that sediments are a significant sink for Pb entering aquatic systems, it is not surprising that rooted macrophytes bioaccumulate significant quantities of the metal. Although there are some similarities to Pb accumulation observed in terrestrial plants (e.g., preferential sequestration of the metal in root tissue), Pb appears to be more bioavailable in sediment than it is in soil. This may be a result of differences in plant physiology between aquatic and terrestrial plants (e.g., more rapid growth or more efficient assimilation of nutrients and ions from a water-saturated medium). While rooted macrophytes are likely to be chronic accumulators of Pb sequestered in sediments, aerial deposition of Pb into aquatic systems may result in pulsed inputs of labile Pb that would be available for uptake by floating macrophytes and algae.

6.4.4.2 Freshwater Invertebrates

Uptake and subsequent bioaccumulation of Pb in freshwater invertebrates varies greatly between species and across taxa as previously characterized in the 2006 Pb AQCD. This section expands on the findings from the 1986 Pb AQCD and 2006 Pb AQCD on bioaccumulation and sequestration of Pb in aquatic invertebrates. In the case of invertebrates, Pb can be bioaccumulated from multiple sources, including the water column, sediment, and dietary exposures, and factors such as proportion of bioavailable Pb, lifestage, age, and metabolism can alter the accumulation rate. In this section, new information on Pb uptake from freshwater and sediments by invertebrates will be considered, followed by a discussion on dietary and water routes of exposure and factors that influence species-specific Pb tissue concentrations such as invertebrate habitat and functional feeding group.

In a recent uptake study in freshwater mussels available since the 2006 Pb AQCD, the Eastern elliptio mussel (*Elliptio complanata*) was shown to accumulate Pb rapidly from water and then reach an equilibrium with exposure level and tissue concentration by two weeks following average daily exposures of 1, 4, 14, 57 or 245 µg Pb/L as Pb nitrate (Mosher et al., 2012). Tissue concentrations of Pb increased at an exposure-dependent rate for the first 14 days and then did not change significantly for the remainder of the 28-day exposure although mussels continued to accumulate Pb. At the end of the exposure period, average Pb in tissue ranged from 0.33 to 898 mg Pb/kg. The authors concluded that the mussels were likely eliminating Pb via pseudo feces and through storage of Pb in shell.

The 2006 Pb AQCD (U.S. EPA, 2006b) summarized studies of uptake of Pb from sediment by aquatic invertebrates and noted that sediment pore water, rather than bulk sediment, is the primary route of exposure. However, a recent study suggests that in the midge, *Chironomus riparius*, total metal concentrations in bulk sediment are better predictors of metal accumulation than dissolved metal concentrations in sediment pore water based on bioaccumulation studies using contaminated sediments from six different sites (Roulier et al., 2008a). Vink (2009) studied six river systems and found that, for a range of metals, uptake by benthic organisms (the oligochaete, Limnodrilus (Family Tubificidae) and the midge, *C. riparius*) from the sediment pore water (as compared with surface water) was observed only occasionally, and solely for Pb. The physiological mechanisms of Pb uptake are still unclear but it is suggested that uptake and elimination of Pb obey different mechanisms than for other heavy metals.

The 2006 Pb AQCD recognized the potential importance of the dietary uptake pathway as a source of Pb exposure for invertebrates. Specifically, in a study with the freshwater amphipod *Hyalella azteca,* dietary exposure was found to contribute to the chronic

toxicity of Pb, while acute toxicity was unaffected (Besser et al., 2004). Since the 2006 Pb AQCD, additional studies have considered the relative importance of water and dietary uptake of Pb in aquatic invertebrates. A stable isotope technique was used to simultaneously measure uptake of environmentally relevant concentrations of Pb (10.4 µg Pb/L) in the water column by the freshwater cladoceran *D. magna* directly from water and through food, the green algae *Pseudokirchneriella subcapitata*. (Komjarova and Blust, 2009a). *D. magna* accumulated the metal from both sources, but the relative proportion of uptake from each source changed over the exposure period. After the first day of exposure, 12% of accumulated Pb was determined to have been absorbed from dietary (algal) sources, but this percentage decreased by day four of exposure to 4%. Pb absorbed from water exposure only resulted in Daphnia body burdens of approximately 62.2 mg Pb/kg dry weight (300 µmol Pb/kg dry weight), and was similar to the amount absorbed by algae (Komjarova and Blust, 2009a). In a comparison of dietary and waterborne exposure as sources of Pb to aquatic invertebrates, no correlation between Pb uptake and dietary exposure was observed in the amphipod *H. azteca (*Borgmann et al., 2007*)*.

Stable isotope analysis was to used measure uptake and elimination simultaneously in net-spinning caddisfly larvae (Hydropsyche sp.) exposed to aqueous Pb concentrations of 0.2 (control) or 0.6 µg Pb/L for 18 days (Evans et al., 2006). The measured uptake constant for Pb in this study was 7.8 g/dry weight per day, and the elimination rate constant of 0.15/day for Pb-exposed larvae was similar in both presence and absence of the metal in the water. Tissue concentrations ranged from approximately 15 to 35 mg Pb/kg. Hydropsychid Pb BCFs ranged from 41 to 65, and averaged 54, indicating a relatively high accumulation when compared to other metals tested (average BCF of 17 for Cd, 7.7 for Cu, and 6.3 for Zn) (Evans et al., 2006).

Recent reports on the tissue distribution of Pb in freshwater organisms generally support the findings of the 2006 Pb AQCD that Pb is primarily sequestered in the gills, hepatopancreas, and muscle. Uptake of Pb by the crayfish *Cherax destructor* exposed to nominal concentration of 5,000 µg Pb/L as Pb nitrate for 21 days resulted in accumulation at the highest concentration in gill, followed by exoskeleton >mid-gut gland >muscle >hemolymph (Morris et al., 2005). Body burden analysis following 96 hour nominal exposure to 50, 100 and 500 µg Pb/L as Pb nitrate in the freshwater snail *Biomphalaria glabrata* indicated that bioaccumulation increased with increasing concentrations of Pb and the highest levels were detected in the digestive gland (Ansaldo et al., 2006).

When the relationship between invertebrate habitat (epibenthic and benthic) and environmental Pb bioaccumulation was investigated, De Jonge et al. (2010) determined

that different environmental fractions of Pb were responsible for invertebrate uptake and exposure. Pb uptake by benthic invertebrate taxa was not significantly correlated to AVS Pb levels, but rather to total sediment concentrations (De Jonge et al., 2009). Conversely, epibenthic invertebrate Pb body burdens were better correlated to AVS concentrations, rather than total Pb sediment concentrations (De Jonge et al., 2010). For instance, the biologically available Pb (e.g., bound to metal-rich granules or metallothioneins) accumulated by the oligochaete *Tubifex tubifex* was determined to correlate with sediment SEM-AVS Pb concentrations (De Jonge et al., 2011). Similarly, Desrosiers et al. (2008) reported that Pb accumulation by chironomid larvae from St. Lawrence river sediments was significantly correlated to both total Pb and reactive Pb sediment concentrations.

Both inter- and intra-specific difference in Pb uptake and bioaccumulation may occur in macroinvertebrates of the same functional-feeding group. Cid et al. (2010) reported significant differences in Pb bioaccumulation between field collected *Ephoron virgo* mayflies and Hydro psyche sp, caddisflies, with only the mayfly exhibiting increased Pb tissue concentrations when collected from Pb-contaminated sites; the caddisfly Pb tissue concentrations were similar between reference and Pb-contaminated areas. The authors also examined the lifestage specific accumulation of Pb for *E. virgo* mayflies, and although there was no statistical difference in Pb tissue concentrations between different lifestages, Pb bioaccumulation did change as mayflies aged (Cid et al., 2010).

Reported BAF values for Pb in aquatic invertebrates from the 2006 Pb AQCD ranged from 499 to 3,670 [Table AX7-2.3.2 (U.S. EPA, 2006c)]. Since the 2006 Pb AQCD, additional BAF values have been established for invertebrates in field studies which tend to be higher than BCF values calculated in laboratory exposures (Casas et al., 2008; Gagnon and Fisher, 1997). A complicating factor in establishing BAF values is that laboratory studies usually assess uptake in water-only or sediment only exposures while field studies take into account dietary sources of Pb as well as waterborne Pb resulting in BAF values that are frequently 100-1,000 times larger than BCF values for the same metal and species (DeForest et al., 2007). The EPA Framework for Metals Risk Assessment states that the latest scientific data on bioaccumulation do not currently support the use of BCFs and BAFs when applied as generic threshold criteria for the hazard potential of metals (U.S. EPA, 2007c). See Section 6.3.3 for further discussion.

As reviewed by Wang and Rainbow (2008) and supported by additional studies reviewed in the present document, there are considerable differences between species in the amount of Pb taken up from the environment and in the levels of Pb retained in the organism. The bioaccumulation and subsequent toxicity of Pb to aquatic organisms (Section 6.4.5) are closely linked to the environmental fate of the metal under variable

environmental conditions (Sections 2.3 and 6.2) as they are highly dependent upon the proportion of free metal ions in the water column.

6.4.4.3 Freshwater Vertebrates

Uptake of Pb by vertebrates considered here includes data from fish species as well as a limited amount of new information on amphibians and aquatic mammals. The bioaccessibility and bioavailability of Pb is affected by abiotic and biotic modifying factors considered in Sections 6.4.2 and 6.4.4. In fish, Pb is taken up from water via the gills and from food via ingestion. Amphibians and aquatic mammals are exposed to waterborne Pb primarily through dietary sources. In the 2006 Pb AQCD, dietary Pb was recognized as a potentially significant source of exposure to all vertebrates since Pb adsorbed to food, particulate matter and sediment can be taken up by aquatic organisms.

Since the 2006 Pb AQCD, tissue accumulation of Pb via gill and dietary uptake has been further characterized in freshwater fish and new techniques such as the use of stable isotopes have been applied to further elucidate bioaccumulation of Pb. For example, patterns of uptake and subsequent excretion of Pb in fish as measured by isotopic ratios of Pb in each tissue can determine whether exposure was due to relatively long term sources (which favor accumulation in bone) or short term sources (which favors accumulation in liver) (Miller et al., 2005). Recent information since the 2006 Pb AQCD, on uptake of Pb by fish from freshwater is reviewed below, followed by studies on dietary uptake as a route of Pb exposure. Next, tissue accumulation patterns in fish species are reported with special consideration of the anterior intestine as a newly identified target of Pb from dietary exposures. Finally, studies that report Pb tissue concentrations in amphibians, reptiles and freshwater mammals are considered.

Freshwater Fish

Pb uptake in freshwater fish is accomplished largely via direct uptake of dissolved Pb from the water column through gill surfaces and by ingestion of Pb-contaminated diets. According to the data presented in the 2006 Pb AQCD (U.S. EPA, 2006b), accumulation rates of Pb are influenced by both environmental factors, such as water pH, DOC, and Ca^{2+} concentrations, and by species-dependent factors, such as metabolism, sequestration, and elimination capacities. The effects of these variables on Pb bioaccumulation in fish are largely identical to the effects observed for invertebrates (discussed above).

Pb in fish is primarily found in bone, gill, blood, kidney and scales (Spry and Wiener, 1991). Since the 2006 Pb AQCD, multiple studies on uptake of Pb from water by fathead

minnow and subsequent tissue distribution have been conducted. Spokas et al. (2006) showed that Pb accumulates to the highest concentration in gill when compared to other tissues over a 24-day exposure. This pattern was also observed in larval fathead minnows exposed to 26 µg Pb/L for 10-30 days, where gill exhibited the highest Pb concentration compared to carcass, intestine, muscle and liver (Grosell et al., 2006a). In the larval minnows, Pb concentration in the intestine exhibited the highest initial accumulation of all tissues on day 3 but then decreased for the remainder of the experiment while concentrations in the other organs continued to increase. By day 30, gill tissue exhibited the highest Pb concentration (approximately 120 mg Pb/kg), followed by whole fish and carcass (whole fish minus gill, liver, muscle and intestine) Pb concentrations (approximately 70 to 80 mg Pb/kg). However, in considering overall internal Pb body burden, nearly 80% was largely concentrated in the bone tissue, while gill contributed <5%.

In another study with fathead minnow, chronic (300 day) exposure to 120 µg Pb/L resulted in accumulation of approximately 41 mg Pb/kg tissue, although this number was decreased from initial body burdens of greater than 104 mg Pb/kg at test initiation (Mager et al., 2010). Tissue distribution at 300 days was consistent with Grosell et al. (2006a) with highest concentration in gill, followed by kidney, anterior intestine, and carcass. Addition of humic acid and carbonate both independently reduced uptake of Pb in these fish over the exposure time period. Interestingly, fathead minnow eggs collected daily during 21 day breeding assays that followed the chronic exposure described above accumulated similar levels of Pb from the test solutions regardless of Pb concentration or water chemistry (e.g., addition of humic acid and carbonate) (Mager et al., 2010). Direct acute exposure from water rather than parental transfer accounted for the majority of the Pb accumulation in eggs. Similarly, exposure of fish to 32.5 µg Pb/L in base water for 150 days resulted in fathead minnow whole body concentrations of approximately 31 mg Pb/kg, with the most rapid accumulation rate occurring within the first 10 days of exposure, followed by an extended period of equilibrium (Mager et al., 2008). In this same study, fish were tested in two additional treatments: 36.7 µg Pb/L in hard water (Ca^{2+} 500 µM) or 38.7 µg Pb/L in humic acid supplemented water (4 mg/L). While the addition of humic acid significantly reduced Pb bioaccumulation in minnows (to approximately 10.4 mg Pb/kg on a whole body basis), Ca^{2+} sulfate did not alter uptake. Despite the fact that Ca^{2+}-mediated Pb toxicity occurred in larval fathead minnow, there was no concurrent effect on whole body Pb accumulation.

Uptake studies in other freshwater teleosts have generally followed the pattern of Pb uptake described above for fathead minnow. In the cichlid, Nile tilapia (*Oreochromis niloticus*), Pb accumulated significantly in gill (45.9 ±34.4 µg/g dry weight at 2,070 µg Pb/L), 57.4 ±26.1 µg/g dry weight at 4,100 µg Pb/L) and liver (14.3 µg/g dry

weight at 2,070 µg Pb/L) and 10.2 µg/g dry weight at 4,100 µg Pb/L) during a 14-day nominal exposure (as Pb nitrate) (Atli and Canli, 2008). In rainbow trout exposed to 100 µg Pb/L (as Pb acetate) for 72 hours, the accumulation in tissues was gill >kidney >liver and this same pattern was observed in all concentrations tested (100-10,000 µg Pb/L) (Suicmez et al., 2006). In contrast to uptake in teleosts, in Pb-uptake studies with the Chondrostei fish Chinese Sturgeon (*Acipenser sinensis*), muscle tissue accumulated higher levels of Pb than gills (Hou et al., 2011).

Sloman et al. (2005) investigated the uptake of Pb in dominant-subordinate pairings of rainbow trout exposed to 46 µg/L or 325 µg Pb/L (as Pb nitrate) for 48 hours. Significant Pb accumulation in gill, liver, and kidney was only observed in the highest concentration. Pb accumulated preferentially in liver of subordinate trout when compared to dominant trout. Brown trout (*Salmo trutta*) exposed to aqueous Pb concentrations ranging from 15 to 46 µg Pb/L for 24 days accumulated 6 mg Pb/kg dry weight in gill tissue and Pb concentrations in liver tissue reached 14 mg Pb/kg dry weight. Interestingly, Pb in gill tissue peaked on day 11 and decreased thereafter, while liver Pb concentrations increased steadily over the exposure period, which may indicate translocation of Pb in brown trout from gill to liver (Heier et al., 2009).

Zebrafish (*Danio rerio*) Pb uptake rates in gills, body (fish without gills) and whole body were determined in fish exposed to water containing 5.2 µg ^{204}Pb/L and sampled at 17, 22, 40 and 48h (Komjarova and Blust, 2009c). This study demonstrated that zebrafish gill tissue is the main uptake site for the metal, as Pb concentrations in these tissues were up to eight times as high as that in other tissues. For example, the rate of Pb uptake in gill tissue was significantly increased from 10 L/kg per hour to 35 L/kg per hour by increasing pH from 6 to 7, and from 20 L/kg per hour to 35 L/kg per hour by increasing Ca^{2+} concentration from 0.1 to 0.5 mM.

The Eurasian silver crucian carp (*Carassius auratus*) collected from a pond containing an average of 1,600 mg Pb/kg in the sediments exhibited increased average Pb whole body burden of 36.5 mg Pb/kg dry weight (range 12 to 68 mg Pb/kg dry weight) (Khozhina and Sherriff, 2008). Pb was primarily sequestered in skin, gill, and bone tissues, but was also detected at elevated levels in muscle and liver tissues, as well as in eggs. Two fish species (*Labeo rohita* and *Ctenopharyngodon idella*) collected from the Upper Lake of Bhopal, India with average Pb concentration 30 µg Pb/L in the water column contained elevated Pb tissue concentrations (Malik et al., 2010). However, while liver and kidney Pb concentrations were similar between the two species (1.5 and 1.1 mg Pb/kg tissue and 1.3 and 1.0 mg Pb/kg tissue for *C. idella* and *L. rohita*, respectively), they accumulated significantly different amounts of Pb in gill and muscle tissues. *C. idella* accumulated

more than twice the Pb in these tissues (1.6 and 1.3 mg Pb/kg) than did *L. rohita* (0.5 and 0.4 mg Pb/kg).

The studies reviewed above generally support the conclusions of the 2006 Pb AQCD (U.S. EPA, 2006b) that the gill is a major site of Pb uptake in fish and that there are species-dependent differences in the rate and pattern of Pb accumulation. As indicated in the 2006 Pb AQCD, exposure duration can be a factor in Pb uptake from water. In a 30-day exposure study, Nile tilapia fingerlings had a three-fold increase in Pb uptake at the gill on day 30 compared to Pb concentration in gill at day 10 and 20 (Kamaruzzaman et al., 2010). In addition to uptake at the gill, a time-dependent uptake of Pb into kidney in rainbow trout exposed to 570 µg Pb/L for 96 hours (Patel et al., 2006) was observed. Pb was accumulated preferentially in the posterior kidney compared to the anterior kidney. A similar pattern was observed by Alves and Wood (2006) in a dietary exposure. In catla (*Catla catla*) fingerlings, the accumulation pattern of Pb was kidney >liver >gill >brain >muscle in both 14 day and 60 day Pb exposures (Palaniappan et al., 2009). In multiple studies with fathead minnow at different exposure durations, tissue uptake patterns were similar at 30 days (Grosell et al., 2006a) and 300 days (Mager et al., 2010). In the larval minnows, Pb concentration in the intestine exhibited the highest initial accumulation of all tissues on day 3 but then decreased for the remainder of the experiment while concentrations in the other organs continued to increase (Grosell et al., 2006a). By day 30, gill tissue exhibited the highest Pb concentration followed by whole fish and carcass (whole fish minus gill, liver, muscle and intestine). The most rapid rate of Pb accumulation in this species occurs within the first 10 days of exposure (Mager et al., 2008). African catfish (*Clarias gariepinus*) exposed to nominal Pb concentrations of 50 to 1,000 µg Pb/L (as Pb nitrate) for 4 weeks accumulated significant amounts of Pb in heart (520-600 mg Pb/kg), liver (150-242 mg Pb/kg), and brain (120-230 mg Pb/kg) tissues (Kudirat, 2008). Doubling the exposure time to 8 weeks increased sequestration of Pb in these tissues as well as in skin (125-137.5 mg Pb/kg) and ovaries (30-60 mg Pb/kg).

Since the 2006 Pb AQCD, several studies have focused on dietary uptake of Pb in teleosts. Metals have been shown to assimilate differently in tissues depending on the exposure route (Rozon-Ramilo et al., 2011; Meyer et al., 2005). Alves et al. (2006) administered a diet of three concentrations of Pb (7, 77 and 520 mg Pb/kg dry weight) to rainbow trout for 21 days. Doses were calculated to be 0.02 µg Pb/day (control), 3.7 µg Pb/day (low concentration), 39.6 µg Pb/day (intermediate concentration),and 221.5 µg Pb/day (high concentration). Concentrations in the study were selected to represent environmentally relevant concentrations in prey. After 21 days exposure to the highest concentration, Pb accumulation was greatest in the intestine, followed by carcass, kidney and liver leading the authors to hypothesize that the intestine is the primary site of exposure in dietary uptake of Pb. All tissues, (gill, liver, kidney, intestine, carcass)

sequestered Pb in a dose-dependent manner. The gills had the greatest concentration of Pb on day 7 (8.0 mg Pb/kg tissue wet weight) and this accumulation decreased to 2.2 mg Pb/kg tissue wet weight by the end of the experiment suggesting that the Pb was excreted or redistributed (Alves et al., 2006). Furthermore, with increasing dietary concentrations, the percentage of Pb retained in the fish decreased. Additionally, in this study red blood cells were identified as a reservoir for dietary Pb. Plasma did not accumulate significant Pb (0.012 mg Pb kg wet weight in the high dose), however, Pb was elevated in blood cells (1.5 mg Pb kg wet weight in the high dose) (Alves et al., 2006).

Additional studies have supported the anterior intestine as a target for Pb in fish. Nile tilapia exposed to dietary Pb for 60 days (105, 418, and 803 mg Pb/kg dry weight) accumulated the greatest concentration of Pb in the intestine, followed by the stomach and then the liver (Dai et al., 2009a). The amount of Pb in tissue increased with increasing dietary Pb concentration. In a 42 day chronic study of dietary uptake in rainbow trout, fish fed 45 or 480 mg Pb/kg, accumulated Pb preferentially in anterior intestine (Alves and Wood, 2006). Pb accumulation in the gut was followed by bone, kidney, liver, spleen, gill, carcass, brain and white muscle (Alves and Wood, 2006). Ojo and Wood (2007) investigated the bioavailability of ingested Pb within different compartments of the rainbow trout gut using an in vitro gut sac technique. Although a significant increase in Pb uptake was observed in the mid-intestines, this was determined to be much lower than Pb uptake rates via gill surfaces. However, given that intestinal uptake rate for Pb did not significantly differ from those derived for essential metals (e.g., Cu, Zn, and Ni), this uptake route is likely to be significant when aqueous Pb concentrations are low and absorption via gill surfaces is negligible (Ojo and Wood, 2007).

Following a chronic 63-day dietary exposure to Pb, male zebrafish had significantly increased Pb body burdens, but did not exhibit any significant impairment when compared with controls. Fish were fed diets consisting of field-collected *Nereis diversicolor* oligochaetes that contained 1.7 or 33 mg Pb/kg dry weight. This resulted in a daily Pb dose of either 0.1 or 0.4 mg Pb/kg (Boyle et al., 2010). At the end of the exposure period, tissue from male fish reared on the high-Pb diet contained approximately 0.6 mg Pb/kg wet weight, as compared with approximately 0.48 mg Pb/kg wet weight in the low-Pb dietary exposure group. Pb level was elevated in female fish fed the high-Pb diet, but not significantly so.

Ciardullo et al. (2008) examined bioaccumulation of Pb in rainbow trout tissues following a 3-year chronic dietary exposure to the metal. Diet was determined to contain 0.19 mg Pb/kg wet weight. Fish skin accumulated the greatest Pb concentrations (0.02 to

0.05 mg Pb/kg wet weight), followed by kidney, gills, liver, and muscle. Pb accumulation in muscles (.005 mg Pb/kg) remained constant over all sampled growth stages (Ciardullo et al., 2008). The authors concluded that dietary Pb was poorly absorbed by rainbow trout. Comparison of dietary and water-borne exposures suggest that although accumulation of Pb can occur from dietary sources, toxicity does not correlate with dietary exposure, but does correlate with gill accumulation from waterborne exposure (Alves et al., 2006). Comparison of uptake rates across the gut and gill have shown that transporter pathways in the gill have a much higher affinity for Pb than do similar pathways in the gut (Ojo and Wood, 2007).

Since the 2006 Pb AQCD, several field studies have considered Pb uptake and bioaccumulation in fish as a tool for environmental assessment. Pb tissue concentrations were elevated in several species of fish exposed in the field to Pb from historical mining waste, and blood Pb concentrations were highly correlated with elevated tissue concentrations, suggesting that blood sampling may be a useful and potentially non-lethal monitoring technique (Brumbaugh et al., 2005).

This review of the recent literature indicates that the primary and most efficient mode of Pb absorption for freshwater fish is assimilation of labile Pb via gill surfaces; recent research indicates that chronic dietary Pb exposure may result in some Pb bioaccumulation although it is not the predominant route of exposure. Nevertheless, if benthic invertebrates comprise a large portion of fish diets in chronically contaminated systems, assimilated Pb loads may be significant. This was demonstrated by Boyle et al. (2010), who showed that laboratory diets consisting of less than one third field-collected Pb-contaminated invertebrates were sufficient to raise fish tissue Pb levels. However, data from field sites suggest that fish accumulation of Pb from dietary sources is highly variable and may be strongly dependent on the physiology of individual species and absorption capacities.

Amphibians

Since the 2006 Pb AQCD, there are a few recent field measurements and laboratory-based studies that consider uptake of Pb in amphibians. Whole body Pb measured in three species of field-collected tadpoles in the Mobile-Tensaw River Delta in Alabama averaged 1.19 mg Pb/kg dry weight in *Rana clamitans*, 0.65 mg Pb/kg dry weight in *Rana catesbeiana* and 1.32 mg Pb/kg dry weight in *Hyla cinerea (*Albrecht et al., 2007*)*. Blood-Pb levels in Ozark hellbender salamanders (*Cryptobranchus alleganiensis bishopi*), a candidate species for the Endangered Species Act, ranged from 0.044 to 0.055 mg/kg dry whole blood weight, in three rivers in Missouri (Huang et al., 2010). In the same study, Pb-blood levels were measured from Eastern hellbenders

(*Cryptobranchus alleganiensis alleganiensis*), a species of concern, collected from four rivers and ranged from 0.075 to 0.088 mg Pb/kg dry whole blood weight.

In a chronic laboratory-based study with tadpoles of the Northern Leopard frog (*Rana pipiens*), Pb tissue concentrations were evaluated following exposures to 3, 10, and 100 µg Pb/L from embryo to metamorphosis. The tadpole tissue concentrations ranged from 0.1 to 224.5 mg Pb/kg dry mass and were positively correlated to Pb concentrations in the water (Chen et al., 2006b). Dose-dependent bioaccumulation of Pb was observed in the livers of tadpoles of the African clawed frog (*Xenopus laevis*) exposed to nominal concentrations ranging from 1.0 to 30,000 µg Pb/L (3 to 115 mg Pb/kg wet weight) for 12 days (Mouchet et al., 2007). Pb concentrations were measured in livers, bodies without liver and whole bodies in Southern leopard frog (*Rana sphenocephala*) tadpoles exposed to Pb in sediment (45 to 7,580 mg Pb/kg dry weight) with corresponding pore water concentrations of 123 to 24,427 µg Pb/L from embryonic stage to metamorphosis (Sparling et al., 2006). There was 100% mortality at 3,940 mg Pb/kg and higher. In all body residues analyzed there was a significant positive correlation between Pb in sediment and Pb in sediment pore water. Concentrations of Pb in liver were similar to results with whole body and bodies without liver indicating that Pb is not preferentially sequestered in liver.

Reptiles

Recent field surveys of Pb in water snakes since the 2006 Pb AQCD indicate that Pb is bioaccumulated in several species. Water snakes spend time in terrestrial and aquatic habitats and could potentially be exposed to atmospherically deposited-Pb in both environments. Average Pb levels in whole body samples of Eastern Ribbon Snakes (*Thamnophis sauritus*) collected from the Mobile-Tensaw River, a large watershed that drains more than 75% of Alabama were 0.35 ± 0.12 mg Pb/kg dry weight) (Albrecht et al., 2007). Burger et al. (2007) measured Pb levels in blood, kidney, liver, muscle and skin from water snakes, (*Nerodia sepedon*) collected from an urban/suburban canal in New Jersey. Pb was highest in skin (0.467 mg Pb/kg wet weight) followed by kidney (0.343 mg Pb/kg wet weight) blood (0.108 mg Pb/kg wet weight), muscle (0.103 mg Pb/kg wet weight) and liver (0.063 mg Pb/kg wet weight). No interspecies differences were observed in blood Pb (range 0.04 to 0.1 mg Pb/kg) from field-collected banded water snakes (*Nerodia fasciata*), brown water snakes (*N. taxispilota*) and cottonmouth (*Agkistrodon piscivorus*) from a reference area and an area contaminated by chemical and radiation releases from the 1950's to the 1980's at the Department of Energy's Savannah River site in South Carolina (Burger et al., 2006). Cottonmouth and brown water snake from the exposed site had significantly higher levels of Pb in tail muscle when compared to the reference creek.

Mammals

Pb bone levels in Eurasian otters (*Lutra lutra*) measured in dead individuals collected in southwest England fell by 73% between 1992 and 2004 (Chadwick et al., 2011). Annual mean bone Pb levels were 446 µg Pb/kg in 1992 and 65 µg Pb/kg in 2004. The 73% decline of Pb in otter bones from 1992 to 2004 was found to coincide with legislative controls on Pb emissions implemented in the U.K. starting in 1986. A positive correlation with stream sediment Pb and bone Pb was also observed in this study. The strength of this correlation decreased with increasing Ca^{2+} in streams.

6.4.4.4 Food Web

In the 2006 Pb AQCD, trophic transfer of Pb through aquatic food chains was considered to be negligible (U.S. EPA, 2006c). Concentrations of Pb in the tissues of aquatic organisms were found to be generally higher in algae and benthic organisms and lower in higher trophic-level consumers indicating that Pb was bioaccumulated but not biomagnified (U.S. EPA, 2006c; Eisler, 2000). Recent literature since the 2006 Pb AQCD, provides evidence of the potential for Pb to be transferred in aquatic food webs. Other studies indicate Pb is decreased with increasing trophic level. This section incorporates recent literature on transfer of Pb through freshwater aquatic food chains including the application of stable isotope techniques to trace the accumulation and dilution of metals through producers and consumers.

Pb was transferred through at least one trophic level in El Niagara reservoir, Aguascalientes, Mexico, a freshwater ecosystem that lacks fishes (Rubio-Franchini et al., 2008). Pb was quantified in sediment (0.55 mg Pb/kg to 21 mg Pb/kg), water (5.8 to 39 µg Pb/L), and zooplankton samples of this freshwater system. BAFs were calculated for predatory and grazing zooplanktonic species. The BAF of the rotifer *A. brightwellii* (BAF 49,300) was up to four times higher than the grazing cladocerans *D. similis* (BAF 9,022) and *M. micrura* (BAF 8,046). According to the authors, since *M. micrura* are prey for *A. brightwellii* this may explain the biomagnifications of Pb observed in the predatory rotifer and provides evidence that Pb biomagnifies at intermediate trophic levels.

The relative contribution of water and food as source of trace metals including Pb was investigated in the larvae of the alderfly *Sialis velata (*Croisetiere et al., 2006*)*. Its prey, the midge (*C. riparius*) was reared in the laboratory and then exposed to trace elements in a metal-contaminated lake for one week prior to being fed to *S. velata*. During the one-week exposure period of *C. riparius* to the contaminated water, five of six trace elements, including Pb, reached steady state within *C. riparius*. Alderfly larvae were held in the lab in uncontaminated lake water and feed one of the treated *C. riparius* per day for up to six

days to measure Pb uptake via prey. A separate group of alderfly larvae were exposed directly to the contaminated lake water for six days and fed uncontaminated *C. riparius* while a third group was exposed to Pb via prey and water. Trace metal concentrations in *S. velata* that consumed contaminated *C. riparius* increased significantly compared to *S. velata* in water-only exposures. Food was concluded to be the primary source of Pb (94%) to these organisms, not Pb in the water.

The trophic transfer of Pb from the sediment dwelling polychaete worm *N. diversicolor* to the invertebrate polychaete predator *Nereis virens* provides additional evidence for assimilation of Pb by a predator and the potential for further transport up the food chain (Rainbow et al., 2006). *N. virens* significantly accumulated Pb from a diet of *N. diversicolor* and there was a significant inverse linear relationship between the trophic transfer coefficient and prey Pb concentration. In the same study, another predator, the decapod *Palaemonetes varians*, did not significantly accumulate Pb from *N. diversicolor* indicating that trophic transfer is dependent on species-specific differences in metal assimilation efficiencies and accumulation patterns.

In a recent dietary metal study, field-collected invertebrates representing ecologically relevant sources of Pb were fed to zebrafish, to assess bioavailability of this metal via food. The polychaete worm *N. diversicolor* was collected from two sites; an estuary contaminated with Pb and a reference site with low metal concentrations (Boyle et al., 2010). Male zebrafish fed Pb-enriched *N. diversicolor* had significant increases in whole-body Pb burden when compared to zebrafish fed prey from the reference site, brine shrimp or flake food diets. There was a trend toward increased Pb levels in females under the same dietary regimen. In this study, deposit feeding invertebrates were shown to mobilize sediment-bound metals in the food chain since zebrafish were exposed only to biologically incorporated metal.

The concentration of Pb in the tissues of various aquatic organisms was measured during the biomonitoring of mining-impacted stream systems in Missouri. Generally, Pb concentrations decreased with increasing trophic level: detritus contained 20 to 60 mg Pb/kg dry weight, while periphyton and algae contained 1 to 30 mg Pb/kg dry weight; invertebrates and fish collected from the same areas exhibited Pb tissue concentrations of 0.1 to 8 mg Pb/kg dry weight (Besser et al., 2007). In addition, Pb concentrations in invertebrates (snails, crayfish, and other benthos) were negatively correlated with Pb concentrations in detritus, periphyton, and algae. Fish tissue concentrations, however, were consistently correlated only with detritus Pb concentrations (Besser et al., 2007).

Other studies have traced Pb in freshwater aquatic food webs and have found no evidence of biomagnification of Pb with increasing trophic level. Watanabe et al. (2008) observed decreasing Pb concentrations through a stream macroinvertebrate food web in Japan from

producers to primary and secondary consumers. In a Brazilian freshwater coastal lagoon food chain, Pb was significantly higher in invertebrates than in fishes (Pereira et al., 2010).

Introduction of exotic species into an aquatic food web may alter Pb concentrations at higher tropic levels. In Lake Erie, the invasive round goby (*Neogobius melanostomus*) and the introduced zebra mussel (*Dreissena polymorpha*) have created a new benthic pathway for transfer of Pb and other metals (Southward Hogan et al., 2007). The goby is a predator of the benthic zebra mussel, while the endemic smallmouth bass (*Micropterus dolomieui*) feed on goby. Since the introduction of goby into the lake, total Pb concentrations have decreased in bass. The authors attribute this decrease of Pb in bass to changes in food web structure, changes in prey contaminant burden or declines in sediment Pb concentrations.

6.4.5 Biological Effects of Pb in Freshwater Systems

This section focuses on the studies of biological effects of Pb on freshwater algae, plants, invertebrates, fish and other biota with an aquatic lifestage (e.g., amphibians) published since the 2006 Pb AQCD. Key studies from the 1977 Pb AQCD, the 1986 Pb AQCD and the 2006 Pb AQCD on biological effects of Pb are summarized where appropriate. Waterborne Pb is highly toxic to aquatic organisms with bioavailability and subsequent toxicity varying depending upon the species and lifestage tested, duration of exposure, the form of Pb tested, and water quality characteristics (e.g., pH, alkalinity, DOC) (Sections 6.4.2 and 6.4.3).

The 2006 Pb AQCD (U.S. EPA, 2006c) noted that the physiological effects of Pb in aquatic organisms can occur at the biochemical, cellular, and tissue levels of biological organization and include inhibition of heme formation, alterations of blood chemistry, and decreases in enzyme levels. A review of the more recent literature corroborated these findings, and added information about induction of oxidative stress by Pb, alterations in chlorophyll, and changes in production and storage of carbohydrates and proteins. Recent studies available since the 2006 Pb AQCD further consider effects of Pb on reproduction and development, growth and survival of aquatic organisms. Alterations to these endpoints can lead to changes at the community and ecosystem levels of biological organization such as decreased abundance, reduced taxa richness, and shifts in species composition (Section 6.1). Effects on reproduction, growth, and survival are reported in additional species with some effects occurring in sensitive freshwater organisms at or near ambient levels of Pb (Table 6-2). Because this review is focused on effects of Pb, studies reviewed for this section include only those for which Pb was the only, or

primary, metal to which the organism was exposed. Areas of research not addressed here include literature related to exposure to Pb from ingestion of shot or pellets. Biological effects of Pb on freshwater algae and plant species are considered below, followed by information on effects on freshwater invertebrates and vertebrates. All reported values are from exposures in which concentrations of Pb were analytically verified unless nominal concentrations are stated.

6.4.5.1 Freshwater Plants and Algae

The toxicity of Pb to algae and plants has been recognized in earlier agency reviews of this metal. In the 1977 Pb AQCD, differences in sensitivity to Pb among different species of algae were observed and concentrations of Pb within the algae varied among genera and within a genus (U.S. EPA, 1977). The 1986 Pb AQCD (U.S. EPA, 1986b) reported that some algal species (e.g., Scenedesmus sp.) were found to exhibit physiological changes when exposed to high Pb concentrations in situ. The observed changes included increased numbers of vacuoles, deformations in cell organelles, and increased autolytic activity. Effects of Pb on algae reported in the 2006 Pb AQCD included decreased growth, deformation, and disintegration of algae cells, and blocking of the pathways that lead to pigment synthesis, thus affecting photosynthesis. Observations in additional algal species since the 2006 Pb AQCD, support these findings and indicate that Pb exposure is associated with oxidative stress. All of these effects were observed at concentrations of Pb that exceed those found currently in most surface waters (Table 6-2).

Recent studies available since the 2006 Pb AQCD, report additional mechanistic information on Pb toxicity to freshwater macrophytes as well as further evidence for effects on oxidative stress and growth endpoints. However, many of these studies were conducted at nominal concentrations of Pb, complicating the comparisons to Pb quantified in surface waters. Furthermore, their relevance to conditions encountered in natural environments is difficult to establish since modifying factors of bioavailability, such as DOC, are often absent from controlled exposures.

The effect of Pb exposure on the structure and function of plant photosystem II was studied in giant duckweed, *S. polyrrhiza (*Ling and Hong, 2009*)*. The Pb concentration of extracted photosystem II particles was found to increase with increasing Pb concentration, and increased Pb concentration was shown to decrease emission peak intensity at 340 nm, amino acid excitation peaks at 230 nm, tyrosine residues, and absorption intensities. This results in decreased efficiency of visible light absorption by affected plants. The authors theorized that Pb^{2+} may replace either Mg^{2+} or Ca^{2+} in

chlorophyll or the oxygen-evolving center, inhibiting photosystem II function through an alteration of chlorophyll structure.

Pb exposure in microalgae species has been linked to several effects, including disruption of thylakoid structure and inhibition of growth in both *Scenedesmus quadricauda* and *Anabaena flos-aquae (*Arunakumara and Zhang, 2008*).* Arunakumara et al. (2008) determined the effect of aqueous Pb on the algal species *S. platensis* using solutions of Pb nitrate. Exposures at 3,440 µg Pb/L stimulated 10-day algal growth; growth was inhibited at higher concentrations of 6,830, 21,800, 32,800 and 44,500 µg Pb/L by 5, 40, 49, and 78%, respectively. In addition to growth inhibition, algal chlorophyll *a* and *b* content were significantly diminished at the three highest Pb exposures (Arunakumara et al., 2008). Although no specific morphological abnormalities were linked to Pb exposure, filament breakage was observed in *S. platensis* at Pb concentrations >50,000 µg Pb/L. Since the 2006 Pb AQCD, the production of reactive oxygen species following Pb exposure has been measured directly in cells of the freshwater algae *Chlamydomonas reinhardtii* at nominal concentrations of Pb as Pb nitrate (0.02 to 52 µg Pb/L) with the greatest response at 3.15 times more stained cells compared to the control sample following an exposure of 2.5 hours (Szivak et al., 2009). Although this study provides direct evidence for a mechanism of Pb-toxicity at the sub-organism level of biological organization, the relevance of the exposure method to conditions encountered in natural environments is unknown. The concentration data are not reliable in this case since Pb concentrations were not quantified and the lowest reported values are below the analytical detection limit for Pb.

At the time of the 1977 Pb AQCD, there was limited information available on Pb effects on aquatic macrophytes. For plants in general, Pb was recognized to affect photosynthesis, mitosis, and growth, however, the majority of studies reporting Pb toxicity were not conducted with plants grown under field conditions (U.S. EPA, 1977). The mechanism for Pb inhibition of photosynthesis was further elucidated in the 1986 Pb AQCD. Additional evidence of Pb effects on plant growth was also observed, however, the available studies were conducted under laboratory conditions at concentrations that exceeded Pb levels in the environment except near smelters or roadsides (U.S. EPA, 1986b). In the 1986 Pb AQCD, EC_{50} values for plant growth were available for several aquatic plants with the lowest EC_{50} of 1,100 µg Pb/L in *Azolla pinnata* exposed to Pb nitrate for 4 days. Effects of Pb on metabolic processes in aquatic plants reviewed in the 2006 Pb AQCD (U.S. EPA, 2006b) included nitrate uptake, nitrogen fixation, ammonium uptake and carbon fixation at concentrations of 20,000 µg Pb/L and higher.

New information is available on Pb effects on oxidative stress endpoints such as changes in antioxidant enzymes, lipid peroxidation, and reduced glutathione in aquatic plant,

algae, and moss species exposed to Pb; however most evidence is from studies with high concentrations where Pb was not quantified in the exposure media. An aquatic moss, *F. antipyretica*, exhibited increased SOD and ascorbate levels following a 2-day exposure to nominal Pb chloride solutions of concentrations of 20, 200, 2,070, 20,700 and 207,200 µg Pb/L. When exposure duration was increased to 7 days, only SOD activity remained significantly increased by Pb exposure (Dazy et al., 2009). Bell-shaped concentration-response curves were commonly observed for the induction of antioxidant enzymes in *F. antipyretica*. The chlorophyll, carotenoid, and protein contents of the aquatic macrophyte *Elodea canadensis* were significantly reduced following Pb accumulation at nominal exposures of 1,000 10,000 and 100,000 µg Pb/L (Dogan et al., 2009). This, along with the induction of some antioxidant systems and the reduction of growth at the highest two exposures, indicated that exposure to the metal caused significant stress, and that toxicity increased with exposure. In addition, native *Myriophyllum quitense* exhibited elevated antioxidant enzyme activity (glutathione-S-transferase, glutathione reductase, and peroxidase) following transplantation in anthropogenically polluted areas containing elevated Pb concentrations. These were correlated with sediment Pb concentrations in the range of 5 to 23 mg Pb/g dry weight (Nimptsch et al., 2005).

Since the 2006 Pb AQCD, toxicity and oxidative stress were also observed in coontail (*C. demersum*) rooted aquatic macrophytes following 7-day nominal exposures to aqueous Pb 200 to 20,700 µg Pb/L, with increasing effects observed with greater exposure concentrations and times. Chlorosis and leaf fragmentation were evident following a 7-day exposure to the highest concentration, while induction of antioxidant enzymes (glutathione, superoxide dismutase, peroxidases, and catalase) was observed at lower exposure concentrations and times. However, as the duration and concentration of Pb exposure was increased, activities of these antioxidant enzymes decreased (Mishra et al., 2006b).

Sobrino et al. (2010) observed reductions in soluble starch stores and proteins with subsequent increases in free sugars and amino acids in *Lemna gibba* plants exposed nominally to Pb (50,000 to 300,000 µg Pb/L); total phenols also increased with increasing Pb exposure. Authors noted that this species exhibited similar responses under extreme temperatures, drought, and disease. According to Odjegba and Fasidi (2006), nominal exposure to 18,600 µg Pb/L as Pb nitrate for 21 days was sufficient to induce a gradual reduction of both chlorophyll and protein content in the macrophyte *Eichhornia crassipes*.

Following 72-hour aqueous exposure to 8,495 µg Pb/L as Pb nitrate, phytochelatin and glutathione concentrations in the freshwater algae *Scenedesmus vacuolatus* were

significantly increased over that of non-exposed algal cultures (Le Faucheur et al., 2006). The 72-hour Pb exposure also significantly reduced *S. vacuolatus* growth, and of all the metals tested (Cu, Zn, Ni, Pb, Ag, As, and Sb), Pb was determined to be the most toxic to the algae species. In the algae *Chlamydomonas reinhardtii*, phytochelatin concentrations were lower than intracellular Pb and not sufficient to bind to accumulated metal following 72-hour exposure (Scheidegger et al., 2011).

In addition to oxidative stress responses, there is new information since the 2006 Pb AQCD on growth effects observed at high concentrations of Pb summarized in Table 6-5. Growth effects at the species level can lead to effects at the population-level of biological organization and higher (Section 6.1.1). Root elongation was significantly reduced in a number of wetland plant species (*Beckmannia syzigachne*, *Juncus effusus*, *Oenanthe javanica*, *Cyperus flabelliformis*, *Cyperus malaccensis*, and *Neyraudia reynaudiana*) following nominal Pb exposures of 20,000 µg Pb/L as Pb nitrate for 21 days (Deng et al., 2009). Lemna sp. aquatic plants were determined to effectively sequester aqueous Pb at nominal exposures of 5,000 and 10,000 µg Pb/L in a 7-day experiment, however, 15,000 µg Pb/L resulted in plant mortality (Hurd and Sternberg, 2008). In another study with duckweed, Paczkowska et al. (2007) observed that nominal Pb exposures of 2,070 to 20,700 µg Pb/L for 9 days stimulated the growth of *Lemna minor* cultures, although there was concurrent evidence of chlorosis and induction of antioxidant enzymes. Additionally, Cd was found to be more toxic than Pb, although the authors determined that this resulted from poor uptake of Pb by *L. minor* (Paczkowska et al., 2007). Pb exposure (as Pb nitrate) caused oxidative damage, growth inhibition, and decreased biochemical parameters, including photosynthetic pigments, proteins, and monosaccharides, in *Wolffia arrhiza* plants. Fresh weight of plants was reduced following both 7- and 14-day exposures to Pb concentrations greater than 2,120 µg Pb/L while chlorophyll *a* content was decreased at 210 µg Pb/L and higher (Piotrowska et al., 2010).

Effects of Pb on algae reported in the 2006 Pb AQCD (U.S. EPA, 2006b) included decreased growth, deformation and disintegration of algae cells, and blocking of the pathways that lead to pigment synthesis, thus affecting photosynthesis. Observations in additional algal species since the 2006 Pb AQCD support these findings. Effects on plants supported by additional evidence in this review and evidence from previous reviews include oxidative damage, decreased photosynthesis and reduced growth. Elevated levels of antioxidant enzymes are commonly observed in aquatic plant, algae, and moss species exposed to Pb. All of the observed effects on aquatic macrophytes and algae occur at concentrations not typically encountered in surface waters of the U.S.

6.4.5.2 Freshwater Invertebrates

Few studies on biological effects of Pb in freshwater invertebrates had been conducted at the time of the 1977 Pb AQCD. One study reported an effect on reproduction in *Daphnia magna* at 30 µg Pb/L (U.S. EPA, 1977). In the 1986 Pb AQCD (U.S. EPA, 1986b), increased mortality was observed in the freshwater snail *Lymnaea palustris* as low as 19 µg Pb/L and reproductive impairment was reported as low as 27 µg Pb/L for Daphnia sp. Population-level endpoints of Pb reviewed in the 2006 Pb AQCD included reproduction, growth, and survival. Pb was recognized to be more toxic in longer-term exposures than shorter-term exposures with chronic toxicity thresholds for reproduction in water fleas (*D. magna*) ranging as low as 30 µg Pb/L. In aquatic invertebrates, Pb has also been shown to affect stress responses and osmoregulation (U.S. EPA, 2006c). Recent evidence that supports previous findings of Pb effects on reproduction and growth in invertebrates is reviewed here as well as limited studies on behavioral effects associated with Pb exposure. Some of these effects are observed in the range of Pb values found in surveys of U.S. surface waters (median 0.50 µg Pb/L, range 0.04 to 30 µg Pb/L), in the U.S. based on a synthesis of NAWQA data reported in the previous 2006 Pb AQCD (U.S. EPA, 2006c) (Table 6-2). The studies are generally presented in this section from responses at the sub-organismal level of biological organization to consideration of endpoints relevant to ecological risk assessment (growth, reproduction, survival).

Recent literature strengthens the evidence indicating that Pb affects enzymes and antioxidant activity in aquatic invertebrates. These alterations at the sub-organismal level may serve as biomarkers for effects at the organism level and higher. In invertebrate species that have hemoglobin, ALAD activity can be measured as a biomarker for Pb exposure. In the freshwater gastropod *B. glabrata* and the freshwater oligochaete *Lumbriculus variegatus* a significant negative correlation between whole body tissue ALAD enzyme activity and increasing Pb was observed following 48-hour exposure to varying nominal concentrations of the metal (Aisemberg et al., 2005). The concentration at which 50% of enzyme inhibition was measured was much lower in *B. glabrata* (23 to 29 µg Pb/L) than in *L. variegatus* (703 µg Pb/L). A significant negative correlation was also observed between ALAD activity and metal accumulation by the organisms. Sodium and potassium ATPase (Na^+/K^+ATPase) activity in gills of Eastern elliptio mussels was significantly reduced following a 28-day exposure to 57 µg Pb/L and 245 µg Pb/L (Mosher et al., 2012). A significant reduction in Na^+ and significant increase in Ca^{2+} in hemolymph was only observed at the highest concentration.

Studies of stress responses to Pb in invertebrates, conducted since the 2006 Pb AQCD, include induction of heat shock proteins and depletion of glycogen reserves. Although these stress responses are correlated with Pb exposure, they are non-specific and may be

altered with exposure to any number of environmental stressors. Induction of heat shock proteins in zebra mussel exposed to an average concentration of 574 µg Pb/L for 10 weeks exhibited a 12-fold higher induction rate as compared to control groups (Singer et al., 2005). Energetic reserves in the freshwater snail *B. glabrata* in the form of glycogen levels were significantly decreased by 20%, 57% and 78% in gonads compared to control animals following 96-hour exposures to nominal concentrations of 50, 100 and 500 µg Pb/L, respectively (Ansaldo et al., 2006). Decreases in glycogen levels were also observed in the pulmonary and digestive gland region at 50 and 100 µg Pb/L treatment levels. Pb did not exacerbate the effects of sustained hypoxia in the crayfish (*C. destructor*) exposed to 5,000 µg Pb/L for 14 days while being subjected to decreasing oxygen levels in water (Morris et al., 2005). The crayfish appeared to cope with Pb by lowering metabolic rates in the presence of the metal.

The effect of Pb on osmoregulatory response has been studied since the 2006 Pb AQCD. The combined effect of Pb and hyperosmotic stress on cell volume regulation was analyzed in vivo and in vitro in the freshwater red crab, *Dilocarcinus pagei* (Amado et al., 2006). Crabs held in either freshwater or brackish water lost 10% of their body weight after one day when exposed to 2,700 µg Pb^{2+}/L as Pb nitrate. This weight loss was transient and was not observed during days 2-10 of the exposure. In vitro, muscle from red crabs exposed to hyperosmotic saline solution had increased ninhydrin-positive substances and muscle weight decreased in isosmotic conditions upon exposure to Pb indicating that this metal affects tissue volume regulation in crabs although the exact mechanism is unknown.

Behavioral responses of aquatic invertebrates to Pb reviewed in the 2006 Pb AQCD (U.S. EPA, 2006b) included avoidance. A limited number of recent studies have considered additional behavioral endpoints. Feeding rate of the blackworm *L. variegatus* was significantly suppressed by day 6 of a 10 day sublethal test in Pb-spiked sediments (Penttinen et al., 2008) as compared to feeding rates at the start of the experiment. However, this decrease of approximately 50% of the initial feeding rate was also observed in the controls; therefore it is likely caused by some other factor other than Pb exposure. Aqueous soil leachates containing multiple metals, including Pb, had no effect on *D. magna* mobility. Authors noted that although some concentrations (13 to 686 µg Pb/L) exceeded Canadian Environmental Quality Guidelines, no significant correlation could be established between Pb exposure and *D. magna* mobility; in fact, the cladocerans were more sensitive to Fe and Al in the leachate than to Pb (Chapman et al., 2010).

Alterations in reproductive and developmental endpoints at the species level can lead to effects at the population-level of biological organization and higher (Section 6.1.1). For

example, reduced fecundity may lead to a decreased population size and developmental defects can compromise the ability of an organism to escape predation. Recent evidence of reproductive and developmental effects of Pb on freshwater invertebrates available since the 2006 Pb AQCD, include data from previously untested species as well as further characterization of reproductive effects in commonly tested organisms such as Daphnia sp (Table 6-5). However, many of these studies are conducted at nominal Pb concentration complicating direct comparison to Pb quantified in freshwater environments. Sublethal concentrations of Pb negatively affected the total number of eggs, hatching success and embryonic survival of the freshwater snail *B. glabrata* exposed to nominal concentrations of 50, 100, or 500 µg Pb/L as Pb nitrate (Ansaldo et al., 2009). Following exposure of adult snails for 96 hours, adults were removed and the eggs were left in the Pb solutions. The total number of eggs was significantly reduced at the highest concentration tested (500 µg Pb/L). Time to hatching was doubled and embryonic survival was significantly decreased at 50 and 100 µg Pb/L, while no embryos survived in the highest concentration. Theegala et al. (2007) observed that the rate of reproduction was significantly impaired in *Daphnia pulex* at >500 µg Pb/L in 21-day exposures at nominal concentrations of Pb. In a 21-day reproductive test in *D. magna* the number of neonates born per female was significantly reduced at nominal concentrations of 25, 250, and 2,500 µg Pb/L (Ha and Choi, 2009). *C. dubia* reproduction was also impacted by a seven-day exposure to 50 to 500 µg Pb/L. Both DOC, and, to a lesser degree, alkalinity were observed to ameliorate the effects of Pb on *C. dubia* reproduction. As DOC increased from 100 µmol C/L to 400 and 600 µmol C/L, the calculated mean EC_{50} values for *C. dubia* reproduction increased from approximately 25 µg Pb/L to 200 µg Pb/L and greater than 500 µg Pb/L, respectively (Mager et al., 2011a). Reproductive variables including average lifespan, rate of reproduction, generation time and rate of population increase were adversely affected in the rotifer *Brachionus patulus* under conditions of increasing turbidity and Pb concentration (García-García et al., 2007).

In larvae of the mosquito, C*ulex quinquefasciatus*, exposed to 50 µg Pb/L, 100 µg Pb/L or 200 µg Pb/L (as Pb nitrate), exposure was found to significantly reduce hatching rate and egg-production at all concentrations and larval emergence rate at 200 µg Pb/L (Kitvatanachai et al., 2005). Larval emergence rates of 78% (F0), 86% (F1) and 86% (F2) were observed in the control group while emergence rates decreased in each generation 46% (F0), 26% (F1) and 58% (F2) in mosquitoes reared in a concentration of 200 µg Pb/L. The time to first emergence also increased slightly to 10 days in the Pb-exposed group as compared to the control group where emergence was first observed on day 9. In the F2 generation of parents exposed to 200 µg Pb/L, the ratio of female to male offspring was 3.6:1.0. No effects were observed on oviposition preference of adult females, larval weight, or larval deformation.

Impacts to growth can lead to effects at the population-level of biological organization and higher (Section 6.1.1). As noted in the 2006 Pb AQCD (U.S. EPA, 2006b), Pb exposure negatively affects the growth of aquatic invertebrates. Some studies reviewed in the previous Pb AQCD suggested that juveniles do not discriminate between the uptake of essential and non-essential metals (Arai et al., 2002). In recent literature (summarized in Table 6-5), the freshwater pulmonate snail *Lymnaea stagnalis* has been identified as a species that is extremely sensitive to Pb exposure. Growth of juveniles was inhibited at EC_{20} <4 µg Pb/L. (Grosell and Brix, 2009; Grosell et al., 2006b). In *L. stagnalis* exposed to 18.9 µg Pb/L for 21 days, Ca^{2+} influx was significantly inhibited and model estimates indicated 83% reduction in growth of newly hatched snails after 30 days at this exposure concentration (Grosell and Brix, 2009). The authors speculate that the high Ca^{2+} demand of juvenile *L. stagnalis* for shell formation and interference of the Ca^{2+} uptake pathway by Pb result in the sensitivity of this species.

In a study of the combined effects of temperature (22 °C or 32 °C), nominal Pb concentration (50, 100 and 200 µg Pb/L as Pb chloride) and presence of a competitor, the population growth rate of two freshwater rotifer species, *Brachionus havanaensis* and *B. rubens*, as measured by quantifying the number of live rotifers for 15 days, responded to presence of stressors (Montufar-Melendez et al., 2007). At the lowest temperature, *B. rubens* suppressed population growth of *B. havanaensis* at 50 µg Pb/L and higher and *B. rubens* population growth did not increase at any Pb concentration at 32 °C, a temperature more suited for *B. havanaensis*. In situ toxicity testing with the woodland crayfish (*Orconectes hylas*) indicated that crayfish survival and biomass were significantly lower in streams impacted by Pb mining and that concentrations of Pb and other metals in water, detritus, macroinvertebrates, fish and crayfish were significantly higher at mining sites (Allert et al., 2009a).

Although Pb is known to cause mortality when invertebrates are exposed at sufficiently high concentrations, species that are tolerant of Pb may not exhibit significant mortality even at high concentrations of Pb. Odonates are highly tolerant of Pb with no significant differences in survival of dragonfly larvae *Pachydiplax longipennis* and *Erythemis simplicicollis* exposed for 7 days to nominal concentrations of Pb as high as 185,000 µg Pb/L (Tollett et al., 2009). This apparent tolerance to Pb may be even more pronounced in natural environments where the presence of multiple modifying factors (e.g., pH, alkalinity, hardness, DOC) influences Pb bioavailability. Other species are more sensitive to Pb in the environment and these responses are reviewed in Section 6.4.6.

6.4.5.3 Freshwater Vertebrates

The 1977 Pb AQCD reported on Pb effects to domestic animals, wildlife and aquatic vertebrates. The available Pb studies were from exposure to Pb via accidental poisoning or ingestion of Pb shot (U.S. EPA, 1977). Studies on aquatic vertebrates reviewed in the 1986 Pb AQCD were limited to hematological, neurological and developmental responses in fish (U.S. EPA, 1986b). In the 2006 Pb AQCD, effects on freshwater vertebrates included recent data for fish specifically considering the effects of water quality parameters on toxicity, as well as limited information on sensitivity of turtles and aquatic stages of frogs to Pb (U.S. EPA, 2006c). Biological effects of Pb on freshwater fish that have been studied since the 2006 Pb AQCD are reviewed here, and limited recent evidence of Pb effects on amphibians are considered. This section presents recent information available on the mechanism of Pb as a neurotoxicant in fish and effects of this metal on blood chemistry. Additional mechanisms of Pb toxicity have been elucidated in the gill and the renal system of fish since the 2006 Pb AQCD. Further supporting evidence of reproductive effects of Pb on fish is discussed along with limited new information on behavioral effects of Pb.

Freshwater Fish

Evidence of toxicity of Pb and other metals to freshwater fish goes back to early observations whereby contamination of natural areas by Pb mining lead to extirpation of fish from streams (U.S. EPA, 1977). At the time of the 1977 Pb AQCD, documented effects of Pb on fish included anemia, mucous secretion, functional damage to inner organs, physical deformities and growth inhibition. Additionally, the role of temperature, pH, hardness and other water quality parameters on Pb toxicity was discussed in the 1977 Pb AQCD. The 1986 Pb AQCD (1986b) reported that hematological and neurological responses were the most commonly observed effects in fish and the lowest exposure concentration causing either hematological or neurological effects was 8 µg Pb/L. These findings were additionally supported in the 2006 Pb AQCD, where observed effects of Pb on fish included inhibition of heme formation, alterations in brain receptors, effects on blood chemistry, and decreases in some enzyme activities (U.S. EPA, 2006c). Functional responses resulting from Pb exposure included increased production of mucus, changes in growth patterns, and gill binding affinities. According to Eisler (2000) and reviewed in the 2006 Pb AQCD, the general symptoms of Pb toxicity in fish include production of excess mucus, lordosis, anemia, darkening of the dorsal tail region, degeneration of the caudal fin, destruction of spinal neurons, ALAD inhibition, growth inhibition, renal pathology, reproductive effects, growth inhibition and mortality.

Evidence of Pb effects on fish available since the 2006 Pb AQCD generally supports the findings in previous Pb reviews and further elucidates the mechanisms of Pb-associated toxicity on some physiological responses. At the sub-organism level, new information on Pb effects on DNA, specific enzymes, ionoregulation and other biochemical responses is presented followed by a discussion of new information on population-level endpoints (i.e., growth reproduction summarized in Table 6-5).

Since the 2006 Pb AQCD evidence of direct interaction of Pb with fish DNA has become available as well as additional studies on the genotoxic effects of Pb exposure to fish. Hong et al. (2007a) observed covalent binding of Pb with kidney DNA from silver crucian carp (*Carassius auratus gibelio*) though extended X-ray absorption fine structure spectroscopy. This study suggests that exposure to Pb results in effects to DNA but the exposure method (in vitro) makes it difficult to estimate the natural environmental conditions that would be equivalent to the experimental one. In the freshwater fish *Prochilodus lineatus*, blood, liver, and gill cells were sampled from fish treated with nominal concentration of 5,000 µg Pb/L as Pb nitrate for 6, 24 and 96-hours and then DNA damage was assessed by comet assay (Monteiro et al., 2011). DNA breaks were observed in all cell types after 96-hour exposure. The concentrations used in this study were high compared to Pb concentrations currently encountered in freshwater (Table 6-2), however, it presents supporting evidence for a possible mechanism of Pb toxicity to fish.

Upregulation of antioxidant enzymes in fish is a well-recognized response to Pb exposure. Since the last review, additional studies demonstrating antioxidant activity as well as evidence for production of reactive oxygen species following Pb exposure are available. Silver crucian carp injected with nominal concentration of 10, 20 or 30 mg Pb/kg wet weight Pb chloride showed a significant increase in the rate of production of superoxide ion and hydrogen peroxide in liver (Ling and Hong, 2010). In the same fish, activities of liver SOD, catalase, ascorbate peroxidase, and glutathione peroxidase were significantly inhibited. Both glutathione and ascorbic acid levels decreased and malondialdehyde content increased with increasing Pb dosage, suggesting that lipid peroxidation was occurring and the liver was depleting antioxidants. Although this exposure pathway is unlikely to be relevant for air related deposition of Pb, it provides evidence for the mechanism of toxicity (production of reactive oxygen species) and the responses of antioxidant enzymes observed in this study are supported by findings in studies from fish from nominal water-only exposures. For example, in the freshwater fish Nile tilapia, liver catalase, liver alkaline phosphatase, Na^+/K^+ATPase, and muscle Ca^{2+}ATPase activities were quantified in various tissues following a 14-day exposure to nominal concentrations (1,000, 2,000 and 4,000 µg Pb/L) of Pb nitrate (Atli and Canli, 2007). Liver catalase activity significantly increased in the 1,000 and 4,000 µg Pb/L

concentrations while liver alkaline phosphatase activity was significantly increased only at the 4,000 µg Pb/L concentration. No significant change in alkaline phosphatase activity was observed in intestine or serum. Ca^{2+}ATPase activity was significantly decreased in muscle. Na^{+}/K^{+}ATPase was elevated in gill in the highest concentration of Pb while all concentrations resulted in significant decreases of this enzyme in intestine. Serum alanine aminotransferase and aspartate aminotransferase activities were elevated in Nile tilapia exposed to 50 µg Pb/L in 4 and 21 day aqueous exposures while elevations in alkaline phosphatase and lactate dehydrogenase were only observed at 21 days (Fırat et al., 2011). In another study with Nile tilapia, Pb had no effect on glutathione measured in liver, gill, intestine, muscle and blood and liver metallothionein levels following a 14-day exposure to 1,000, 2,000 and 4,000 µg Pb/L concentrations of Pb as Pb nitrate (Atli and Canli, 2008).

Metabolic enzyme activity in teleosts has also been measured following dietary exposures. Alves and Wood (2006) in a 42-day chronic dietary Pb study with 45 and 480 mg Pb/kg found that gill Na^{+}/K^{+}ATPase activity was not affected in rainbow trout while increased Na^{+}/K^{+}ATPase was observed in the anterior intestine. Metabolic activities measured in liver and kidney of Nile tilapia following 60-day dietary administration of 100, 400, and 800 mg Pb/kg indicated that alanine transaminase, aspartate transaminase, and lactate dehydrogenase activities significantly decreased in kidney in a concentration-dependent manner (Dai et al., 2009b) and increased in liver with increasing concentration of dietary Pb. In a subsequent study using the same exposure paradigm, the digestive enzymes amylase, trypsin and lipase in tilapia were inhibited by dietary Pb in a concentration-dependent manner (Dai et al., 2009a). Lesions were also evident in histological sections from livers of Pb-exposed fish from this study and included irregular hepatocytes, cell hypertrophy, and vacuolation although no quantification of lesions by dose-group was presented.

There is also evidence for Pb exposure leading to changes in hepatic CYP450 content although relevance of these in vitro and injection studies to air related exposures to Pb is unknown. Pb was shown to inhibit hepatic cytochrome P450 in vitro in carp (*C. carpio*), silver carp (*Hypothalmichtys molitrix)* and wels catfish (*Silurus glanis*) in a concentration-dependent manner from 0 to 4 µg/mL (Pb^{2+}) (Henczova et al., 2008). The concentrations of Pb that resulted in 50% inhibition of EROD and 7-ethoxycoumarin-o-deethylase (ECOD) isoenzymes varied with the fish species. Silver carp was the least sensitive to the inhibitory effects of Pb (EROD 1.21, ECOD 1.52 µg Pb/mL) while carp EROD activity was inhibited at 0.76 µg Pb/mL. Interaction of Pb with cytochrome P450 was verified by spectral changes using Fourier Transform Infrared (FTIR) spectroscopy. In the same study, CYP450 content was elevated and EROD isoenzyme activities were decreased in vivo in silver carp for two days following an injection of 2 mg Pb/kg as

Pb acetate and returned to control values by 6 days. Liver damage to African catfish exposed to nominal concentrations of Pb (50-1,000 µg Pb/L) for 4 or 8 weeks included hepatic vacuolar degeneration followed by necrosis of hepatocytes (Adeyemo, 2008b). The severity of observed histopathological effects in the liver was proportional to the duration of exposure and concentration of Pb.

In environmental assessments of metal-impacted habitats, ALAD is a recognized biomarker of Pb exposure (U.S. EPA, 2006c). For example, lower ALAD activity has been significantly correlated with elevated blood Pb concentrations in wild caught fish from Pb-Zn mining areas although there are differences in species sensitivity (Schmitt et al., 2007b; Schmitt et al., 2005). Suppression of ALAD activity in brown trout transplanted to a metal contaminated stream was linked to Pb accumulation on gills and in liver in a 23-day exposure (Heier et al., 2009). Alves Costa et al. (2007) observed inhibition of ALAD in hepatocytes of the neotropical traira (*Hoplias malabaricus*) following dietary dosing of 21 mg Pb/kg every 5 days for 70 days. Cytoskeletal and cytoplasmic disorganization were observed in histopathological examination of affected hepatocytes. In fathead minnow exposed to Pb in either control water (33 µg Pb/L), $CaSO_4$ (37 µg Pb/L) or (39 µg Pb/L) humic acid-supplemented water for 30 days and subsequently analyzed by quantitative PCR analysis there were no significant changes in ALAD mRNA gene response leading the authors to speculate that water chemistry alone does not influence this gene response (Mager et al., 2008). In the same study, glucose-6-phosphate dehydrogenase, glutathione-S-transferase and ferritin were upregulated, in microarray analysis; however, no changes in whole body ion concentrations were observed (Mager et al., 2008).

In fish, changes in blood chemistry associated with Pb exposure were noted in the 2006 Pb AQCD (U.S. EPA, 2006b), however, only limited recent studies consider effects on blood parameters. In a 70-day feeding study with traira exposed to dietary doses (21 mg Pb/kg as Pb nitrate via prey [Astyanax sp.]) each five days (corresponding to daily nominal doses of approximately 4 mg Pb/kg), there were no significant changes to leukocytes or hemoglobin concentration and volume (Oliveira Ribeiro et al., 2006). Significant differences in area, elongation, and roundness of erythrocytes were observed in the Pb-exposed individuals using light microscopy image analysis. Other studies available since the 2006 Pb AQCD have only shown effects on blood chemistry at high aqueous concentrations of Pb that are not representative of Pb concentrations in U.S. surface waters. For example, in the African catfish packed cell volume decreased with increasing nominal concentration of Pb (25,000 to 200,000 µg Pb/L as Pb nitrate) and platelet counts increased in a 96-hour exposure (Adeyemo, 2007). Red blood cell counts also decreased in some of the treatments when compared to controls, although the response was not dose-dependent and so may not have been caused by Pb exposure.

Disruption of ionoregulation is one of the major modes of action of Pb toxicity. The gill has long been recognized as a target of Pb in teleosts. Acute Pb toxicity at the fish gill primarily involves disruption of Ca^{2+} homeostasis as previously characterized in the 2006 Pb AQCD (Rogers and Wood, 2004; Rogers and Wood, 2003). In addition to this mechanism, Pb was found to induce ionoregulatory toxicity at the gill of rainbow trout through a binding of Pb with Na^{+}/K^{+}ATPase and rapid inhibition of carbonic anhydrase activity thus enabling noncompetitive inhibition of Na^{+} and Cl^{-} influx (Rogers et al., 2005). Alves et al. (2006) administered a diet of three concentrations of Pb (7, 77 and 520 mg Pb/kg dry weight) to rainbow trout for 21 days, and measured physiological parameters including Na^{+} and Ca^{2+} influx rate from water. Dietary Pb had no effect on brachial Na^{+} and Ca^{2+} rates except on day 8 where Na^{+} influx rates were significantly elevated. These studies suggest that Pb is intermediate between purely Ca^{2+} antagonists such as Zn^{2+} and Cd^{2+} and disruptors of Na^{+} and Cl^{-} balance such as Ag^{+} and Cu^{2+}. This finding has implications for BLM modeling since it suggests that both Ca^{2+} and Na^{+} need to be considered as protective cations for Pb toxicity. Indeed, protection from Pb toxicity by both Na^{+} and Ca^{2+} has been documented in freshwater fish (Komjarova and Blust, 2009b).

Additional experiments conducted since the 2006 Pb AQCD provide supporting evidence for underlying mechanisms of Pb toxicity. It was previously established that long-term exposures of Pb can impact gill structure and function. Histopathological observations of gill tissue in the catfish (*C. gariepinus*) following an 8-week aqueous exposure to nominal concentrations of Pb nitrate revealed focal areas of epithelial hyperplasia and necrosis at the lower exposure concentrations (50 µg Pb/L and 100 µg Pb/L) (Adeyemo, 2008a). Hyperplasia of mucous cells and epithelial cells were apparent in the tissue from fish exposed the highest concentrations of Pb in the study (500 µg Pb/L and 1,000 µg Pb/L). In vitro incubation of gill tissue from fathead minnow with Pb concentrations of 2,500, 12,500 and 25,000 µg Pb/L for 60 minutes decreased the ratio of reduced glutathione to oxidized glutathione, indicating that lipid peroxidation at the gill likely contributes to Pb toxicity at low water hardness (Spokas et al., 2006). It is difficult to extrapolate these observations to natural environments due to the methods used for exposure and the use of nominal exposure concentrations.

In addition to recent evidence of Pb interruption of Na^{+} and Cl^{-} at the gill (Rogers et al., 2005), Pb can interfere with the ionoregulation of Na^{+} and Cl^{-} and tubular reabsorption of Ca^{2+}, Mg^{2+}, glucose, and water in the teleost kidney (Patel et al., 2006). Renal parameters including urine flow rate, glomerular filtration rate, urine pH, and ammonia excretion were monitored in a 96-hour exposure of rainbow trout to analytically verified concentration of 1,200 µg Pb/L as Pb nitrate. Rates of Na^{+} and Cl^{-} excretion decreased by 30% by 48 hours while Mg excretion increased two-to-three fold by 96 hours. Urine flow

rate was not altered by Pb exposure, although urinary Pb excretion rate was significantly increased. After 24 hours of Pb exposure, the urine excretion rate of Ca^{2+} increased significantly by approximately 43% and remained elevated above the excretion rate in the control group for the duration of the exposure. Glomerular filtration rate significantly decreased only during the last 12 hours of the exposure. Ammonia excretion rate increased significantly at 48 hours as urine pH correspondingly decreased. At the end of the experiment glucose excretion was significantly greater in Pb-exposed fish. Although the exposures in this study approached the 96-hour LC_{50}, nephrotoxic effects of Pb indicate the need to consider additional binding sites for this metal in the development of biotic ligand modeling (Patel et al., 2006). Additional evidence for Pb effects on ion levels were observed in serum of Nile tilapia; Na^+ and Cl^- were decreased and K^+ levels were elevated following a 21 day nominal exposure to 50 µg Pb/L as Pb nitrate (Fırat et al., 2011).

Neurological responses of fish to Pb exposure were reported in the 1986 Pb AQCD (U.S. EPA, 1986b). Additional evidence of the neurotoxic effects of Pb on teleosts has become available since the 2006 Pb AQCD. The mitogen-activated protein kinases (MAPK), extracellular signal-regulated kinase (ERK)1/2 and $p38^{MAPK}$ were identified for the first time as possible molecular targets for Pb neurotoxicity in a teleost (Leal et al., 2006). The phosphorylation of ERK1/2 and $p38^{MAPK}$ by Pb was determined in vitro and in vivo in the catfish (*Rhamdia quelen*). *R. quelen* exposed to a nominal concentration of 1,000 µg Pb/L (as Pb acetate) for two days showed a significant increase in phosphorylation of ERK1/2 and $p38^{MAPK}$ in the nervous system. Incubation of cerebellar slices for 3 hours in 1,035 and 2,070 µg Pb/L as Pb acetate also showed significant phosphorylation of MAPKs. The observed effects of Pb on the MAPK family of signaling proteins have implications for control of brain development, apoptosis and stress response. In the neotropical fish traira, muscle cholinesterase was significantly inhibited after 14 dietary doses of 21 mg Pb/kg wet weight (Rabitto et al., 2005). Histopathological observations of brains of African catfish exposed to nominal concentrations of 500 µg Pb/L or 1,000 µg Pb/L Pb as Pb nitrate for 4 weeks included perivascular edema, focal areas of malacia, and diffuse areas of neuronal degeneration (Adeyemo, 2008b). As in the observed effects of Pb on gill function and ionoregulation, it is difficult to assess the significance of these findings to fish in natural environments due to the methods used for exposure.

Evidence from the 2006 Pb AQCD (U.S. EPA, 2006b) and earlier Pb reviews indicate that Pb can impair both cognitive and motor function in fish. Reduced locomotion and foraging ability were observed in Chinese sturgeon juveniles exhibiting abnormal body curvature following nominal exposure to either 800 or 1,600 µg Pb/L for 112 days (Hou et al., 2011). Actual concentrations of Pb were quantified at the end of the 112-day exposure period (30 to 50% of test media was renewed daily): 129 µg Pb/L (200 µg Pb/L,

nominal), 458 µg Pb/L (800 µg Pb/L nominal), and 1,276 µg Pb/L (1,600 µg Pb/L, nominal). These chondrostean fish gradually recovered from deformities during a depuration period and were able to swim and forage effectively 6 weeks after transfer into clean water

Since the 2006 Pb AQCD, several studies integrating behavioral and physiological measures of Pb toxicity have been conducted on fish. Some of these observations are reported to occur at concentrations of Pb reported in freshwater. Zebrafish embryos exposed nominally to low concentrations of Pb as Pb chloride (2.0 and 6.0 µg Pb/L prepared from serial dilutions of a stock solution) until 24 hours post-fertilization and then subsequently tested as larvae or adult fish exhibited behavioral disruptions in response to mechanosensory and visual stimuli (Rice et al., 2011). Although Pb was not measured in the water, Pb uptake in the embryos was quantified during the first 24 hours post-fertilization (approximately 0.08 nM/100 embryos at 2.0 µg Pb/L and 0.32 nM Pb/100 embryos at 6.0 µg Pb/L). Startle response time in larvae measured as maximum head turn velocity and escape time decreased in a concentration-dependent pattern following a directional, mechanical stimulus (tapping). The pattern of escape swimming was altered in larvae of Pb-exposed embryos compared to controls. In the adult fish hatched from Pb-exposed embryos (6.0 µg Pb/L), visual response to a rotating black bar against a white background (ability to detect contrast) was significantly degraded. These findings provide evidence for behavioral effects of Pb at concentrations lower than previously reported in fish (U.S. EPA, 2006c), however, aqueous exposure concentrations were not analytically verified.

Sloman et al. (2005) investigated the effect of Pb on hierarchical social interactions and the corresponding monoaminergic profiles in rainbow trout. Trout were allowed to establish dominant-subordinate relationships for 24 hours, and then were exposed to 46 µg Pb/L or 325 µg Pb/L (Pb nitrate) for 48 hours to assess effects on behavior and brain monoamines. In non-exposed fish, subordinate individuals had higher concentrations of circulating plasma cortisol and telencephalic 5-hydroxyindoleacetic acid/5-hydroxytryptamine (serotonin) (5-HIAA/5-HT) ratios. In the high concentration of Pb, there was significant uptake of Pb into gill, kidney and liver when compared with the control group and dominant fish appeared to have elevated hypothalamic 5-HIAA/5HT ratios. Uptake of Pb into the liver was higher in subordinate fish when compared to the dominant fish. No significant differences were observed in cortisol levels or behavior after metal exposure.

Mager et al. (2010) conducted prey capture assays with 10 day old fathead minnow larvae born from adult fish exposed to 120 µg Pb/L for 300 days, then subsequently tested in a breeding assay for 21 days. The time interval between 1st and 5th ingestion of

10 prey items (*Artemia nauplii*) was used as a measure of behavior and motor function of offspring of Pb-exposed fish. Larvae were offered 10 Artemia and the number ingested within 5 minutes was scored. The number of larvae ingesting 5 Artemia decreased within the time period in offspring of Pb-exposed fish as compared to the control group, leading the authors to suggest this behavior is indicative of motor/behavioral impairment. In another study with fathead minnows, swimming performance measured as critical aerobic swim speed was significantly impaired in minnows in 24-hour acute (139 µg Pb/L) and chronic 33 to 57 day (143 µg Pb/L) exposures, however, no significant difference in swim speed was observed in chronic exposures to 33 µg Pb/L (Mager and Grosell, 2011).

Alterations in reproductive and developmental endpoints at the species level can lead to effects at the population-level of biological organization and higher (Section 6.1.1). For example, reduced fecundity may lead to a decreased population size and developmental effects may decrease the ability of a fish to escape predators or reduce spawning mobility. Reproductive and developmental effects of Pb in fish have been reported for several decades. In the 1977 Pb AQCD, second generation brook trout (*Salvelinus fontinalis*) exposed to 235 or 474 µg Pb/L were shown to develop severe spinal deformities (lordoscoliosis) (U.S. EPA, 1977). Pb concentration of 120 µg Pb/L produced spinal curvature in rainbow trout (*Oncorhynchus mykiss*) and spinal curvatures were observed in developing eggs of killifish as reviewed in the 1986 Pb AQCD (U.S. EPA, 1986b). Recent studies on reproductive effects of Pb in fish from oocyte formation to spawning are summarized in Table 6-5.

Reproductive performance of zebrafish as measured by incidence of spawning, numbers of eggs per breeding pair or hatch rate of embryos was unaffected following a 63 day diet of field-collected Pb-contaminated polychaetes that were representative of a daily dose of 0.3-0.48 mg Pb/kg per day (dry weight diet/wet weight fish) through food (Boyle et al., 2010). Mager et al. (2010) conducted 21-day breeding exposures at the end of chronic 300 day toxicity testing with fathead minnow. Non-exposed breeders were switched to water containing Pb and Pb-exposed breeders were moved to control tanks and effects on egg hatchability and embryo Pb accumulation were assessed. Fish in the high Pb concentration with HCO_3^- (113 µg Pb/L) and DOC (112 µg Pb/L) and the low Pb concentration with HCO_3^- (31 µg Pb/L) reduced total reproductive output, while a significant increase in average egg mass was observed in the high Pb HCO_3^- and DOC treatments as compared to egg mass size in controls and in low HCO_3^- and DOC treatments with Pb. No significant differences were present between treatments in egg hatchability.

The effects of metals on embryonic stage of fish development in *C. carpio* and other species were reviewed in Jezierska et al. (2009) and included developmental

abnormalities during organogenesis as well as embryonic and larval malformations. The authors concluded that the initial period of embryonic development, just after fertilization, and the period of hatching are the times at which developing embryos are most sensitive to metals. Additional nominal exposure studies provide supporting evidence for embryo malformations associated with Pb-exposure. A significant concentration-dependent increase in morphological malformations was observed in African catfish embryos exposed to nominal concentrations of 100 µg Pb/L, 300 µg Pb/L or 500 µg Pb/L Pb nitrate from 6 hours post-fertilization to 168 hours post-fertilization (Osman et al., 2007b). Hatching was delayed with increasing Pb concentration and hatch success of the embryos decreased from 75% in the controls to 40% in the group exposed to 500 µg Pb/L. Chinese sturgeon exposed to nominal concentrations of 200 µg Pb/L, 800 µg Pb/L or 1,600 µg Pb/L for 112 days (96 hour post-fertilized eggs through juvenile stages) exhibited body curvatures in the two highest concentrations (Hou et al., 2011). During a 42 day depuration period in clean water following exposure, the degree of curvature in affected individuals decreased with decreasing tissue concentrations of Pb.

Reproductive and endocrine effects of Pb have also been reported at the cellular level in fish, including alterations in gonadal tissue and hormone secretions that are associated with Pb-exposure, however, recent studies that report these effects are limited to experiments where only nominal concentrations of Pb were tested. Histopathological observations of ovarian tissue in the African catfish following an 8-week aqueous exposure to Pb nitrate indicated necrosis of ovarian follicles at the lowest concentration tested (50 µg Pb/L) (Adeyemo, 2008a). Severe degeneration of ovarian follicles was observed in the highest concentrations of 500 µg Pb/L and 1,000 µg Pb/L. Chaube et al. (2010) considered the effects of Pb on steroid levels through 12 and 24 hour in vitro exposures of post-vitellogenic ovaries from the catfish (*Heteropneustes fossilis*) to nominal concentrations of Pb as Pb nitrate (0, 10, 100, 1,000, 3,000 and 10,000 µg Pb/L). Progesterone, 17-hydroxyprogesterone, 17, 20 beta-dihydroxyprogesterone, corticosterone, 21-deoxycortisol and deoxycorticosterone were inhibited in a dose-dependent manner. Pb was stimulatory on the steroids estradiol-17-β, testosterone and cortisol at low concentrations, and inhibitory at higher concentrations. The authors propose that the disruption of steroid production and altered hormone secretion patterns observed at the lower concentrations of Pb in this study are suggestive of the potential for impacts to fish reproduction (Chaube et al., 2010).

There is also evidence for alterations in steroid levels associated with Pb exposure in other species of fish although these studies were all conducted with nominal concentrations of Pb and the actual exposure concentrations were not verified. Carp (*Cyprinus carpio*) exposed for 35 days to nominal concentration of 410 µg Pb/L experienced altered plasma cortisol and prolactin levels. Plasma cortisol levels

significantly increased throughout the study period while plasma prolactin increased up to day 14 and then declined and was not significantly different from controls by the end of the experiment (Ramesh et al., 2009). Cortisol levels were significantly decreased in Nile tilapia exposed to 50 µg Pb/L (nominal) for 4 days but were followed by a return to control levels at 21 days of exposure (Fırat et al., 2011). In a comparative study between in vitro and in vivo estrogenic activity of Pb, vitellogenin was reported to be significantly induced in juvenile goldfish (*Carassius auratus*) following 96-hour exposure to nominal concentration of 0.2 and 0.02 µg Pb/L when compared to control fish (Isidori et al., 2010). In the same study, estrogenicity of Pb was detected in vitro using a proliferation assay with estrogen receptor-positive human MCF-7 cells. The estrogenic effects of Pb reported by the authors were observed at concentrations at or below that of Pb typically encountered in freshwaters, however, actual concentrations of Pb were not measured and the reported concentrations were at or below analytical detection limits for Pb. The observations of effects of Pb on vitellogenin are interesting; however, additional studies are warranted considering the difficulty in maintaining these low concentrations of Pb. The relevance of the observed in vitro activity to air related exposure to Pb in natural environments is unknown.

Reduction of growth in fish was noted as an effect of Pb exposure in the 2006 Pb AQCD. Recent studies available since the 2006 Pb AQCD do not present consistent evidence of growth reduction in fish associated with Pb (Table 6-5). In a series of exposures in which Ca^{2+}, DOC and pH were varied to assess effects on Pb toxicity to fathead minnows, Grosell et al. (2006a) observed a significant increase in growth in some groups exposed to higher concentrations, however, the increase in body mass was noted to have occurred in tanks with high mortality earlier in the exposure (Grosell et al., 2006a). Fathead minnows exposed to 33 µg Pb/L to test swimming performance had significantly greater body length and body mass compared to control fish following a mean Pb exposure duration of 41 days (range 33 to 57 days) (Mager and Grosell, 2011). In 30 day chronic tests in which a range of pH values (6.4, 7.5 and 8.3) were tested with low (25-32 µg Pb/L), intermediate (82-156 µg Pb/L) and high (297-453 µg Pb/L) concentrations of Pb, Mager et al. (2011b) did not observe growth impairment in fathead minnows at environmentally relevant concentrations of Pb. However, two 60-day early lifestage tests with rainbow trout showed differences in LOEC for reduced growth (Mebane et al., 2008). In the first test, a 69-day exposure, the LOECs for mortality and reduced growth were the same (54 µg Pb/L). In the second test, a 62-day exposure of Pb to rainbow trout, the LOEC for fish length was 18 µg Pb/L with an EC_{20} of >87 µg Pb/L.

No effects on growth were observed in recently conducted feeding studies with fish. Growth and survival were not significantly affected in juvenile rainbow trout, fathead minnow and channel catfish (*Ictalurus punctatus*) fed a live diet of *L. variegatus*

contaminated with Pb (846-1,000 µg Pb/L per g dry mass for 30 days). (Erickson et al., 2010). No effects on growth rates were observed in rainbow trout administered a diet containing three concentrations of Pb (7, 77 and 520 mg Pb/kg dry weight) for 21 days (Alves et al., 2006) or in Nile tilapia fed diets with nominal concentration of 100, 400, or 800 mg/kg Pb dry weight for 60 days (Dai et al., 2009b).

In one recent field study, faster growth rates were associated with lower whole-body trace element concentrations in salmon (*Salmo salar*) across several streams in New Hampshire and Massachusetts, U.S., regardless of whether accumulation was from prey items or from water (Ward et al., 2010). In sites where conditions in the streams were conducive to rapid salmon growth, Pb concentrations were 86% lower than in streams where salmon were smaller.

Amphibians

Amphibians move between terrestrial and aquatic habitats and can therefore be exposed to Pb both on land and in water. The studies reviewed here are all aquatic or sediment exposures. Biological effects of Pb on amphibians in terrestrial exposure scenarios are reviewed in Sections 6.3.3.3 and 6.3.4.3. Amphibians lay their eggs in or around water making them susceptible to water-borne Pb during swimming, breeding and development. In the 2006 Pb AQCD amphibians were considered to be relatively tolerant to Pb. Observed responses to Pb exposure included decreased enzyme activity (e.g., ALAD reduction) and changes in behavior summarized in Table AX7-2.4.3 (U.S. EPA, 2006c). Since the 2006 Pb AQCD, studies conducted within two orders of magnitude of the range of published Pb concentrations for surface waters and sediments of the U.S. (Section 6.2.3) have indicated sublethal effects on tadpole endpoints including growth, deformity, and swimming ability. Genotoxic and enzymatic effects of Pb following chronic exposures have been assessed in laboratory bioassays; however, these studies were limited to nominal exposures.

The genotoxic potential of Pb to larvae of the frog (*X. laevis*) was assessed by determining the number of micronucleated erythrocytes per thousand (MNE) following a 12-day exposure to nominal concentrations of Pb as Pb nitrate (Mouchet et al., 2007). The lowest Pb concentrations with *X. laevis* (10 and 100 µg Pb/L) did not exhibit genotoxic effects while both 1,000 and 10,000 µg Pb/L significantly increased MNE to 14 and 202, respectively compared to the control (6 MNE). In another chronic genotoxic study, erythrocytic micronuclei and erythrocytic nuclear abnormalities were significantly increased with increasing Pb concentrations (700 µg Pb/L, 1,400 µg Pb/L, 14,000 µg Pb/L, 70,000 µg Pb/L) during 45, 60, and 75-day exposures of tadpoles *Bufo raddei (*Zhang et al., 2007b*)*. The authors noted that the erythrocytic micronuclei and

erythrocytic nuclear abnormalities frequencies generally decreased with increasing exposure time and that this may be indicative of regulation of genotoxic factors by tadpoles.

Endpoints of oxidative damage were measured in testes of the black-spotted frog (*Rana nigromaculata*) treated with nominal concentrations of 100 µg Pb/L, 200 µg Pb/L, 400 µg Pb/L, 800 µg Pb/L or 1,600 µg Pb/L Pb nitrate by epidermal absorption for 30 days (Wang and Jia, 2009). All doses significantly increased MDA, a product of oxidative stress, and glutathione levels were elevated in all but the lowest treatment group. In the same study, damage to DNA assessed by DNA tail length showed effects at >200 µg Pb/L and DNA tail movement showed effects at >400 µg Pb/L. The authors concluded that the effects on endpoints of oxidative stress and DNA damage detected in testes indicated a possible reproductive effect of Pb to black-spotted frogs. The exposure method and use of nominal concentration in this study make it difficult to determine the relevance of this study to exposure scenarios under natural environmental conditions.

Various sublethal endpoints (growth, deformity, swimming ability, metamorphosis) were evaluated in northern leopard frog (*R. pipiens*) tadpoles exposed to nominal concentrations of 3, 10, and 100 µg Pb/L as Pb nitrate from embryonic stage to metamorphosis (Chen et al., 2006b). In this chronic study, the concentrations represent the range of Pb found in surface freshwaters across the U.S. The lowest concentration of 3 µg Pb/L approaches the EPA chronic criterion for Pb of 2.5 µg Pb/L at a hardness of 100 mg/L or 4.5 µg Pb/L at a hardness of 170 mg/L (U.S. EPA, 2002b). No effects were observed in the lowest concentration. In the 100 µg Pb/L treatment, tadpole growth rate was slower (Gosner stages 25-30), 92% of tadpoles had lateral spinal curvature (compared with 6% in the control) and maximum swimming speed was significantly slower than the other treatment groups. In this study, Pb concentrations in the tissues of tadpoles were quantified and the authors reported that they were within the range of reported tissue concentrations from wild-caught populations.

The effects of Pb-contaminated sediment on early growth and development were assessed in the southern leopard frog (Sparling et al., 2006). Tadpoles exposed to Pb in sediment (45, 75, 180, 540, 2,360, 3,940, 5,520, and 7,580 mg Pb/kg dry weight) with corresponding sediment pore water concentrations of 123, 227, 589, 1,833, 8,121, 13,579, 19,038 and 24,427 µg Pb/L from embryonic stage to metamorphosis exhibited sublethal responses to Pb in sediment at levels below 3,940 mg Pb/kg. There was 100% mortality in the 3,940, 5,520 and 7,580 mg Pb/kg exposures by day 5. The authors noted that the most profound effects of Pb on the tadpoles were on skeletal development. At 75 mg Pb/kg, subtle effects on skeletal formation such as clinomely and brachydactyly were observed. Skeletal malformations increased in severity at 540 mg Pb/kg and included

clinodactyly, brachymely and spinal curvature and these effects persisted after metamorphosis. At the highest concentration with surviving tadpoles (2,360 mg Pb/kg) all individuals displayed severe skeletal malformations that impacted mobility. Other sublethal effects of Pb observed in this study were reduced rates of early growth of tadpoles at concentrations ≤ 540 mg Pb/kg and increased time to metamorphosis in the 2,360mg Pb/kg (8,121µg Pb/L sediment pore water) treatment.

Birds

As reviewed in Koivula and Eeva (2010) measurement of enzymes associated with oxidative stress in birds is a well-established biomarker of exposure to metals, however, little is known about the effects of this stress response in wild populations or at higher levels of ecological organization. Changes in ALAD activity and other oxidative stress biomarkers at low levels of Pb exposure were recently documented in mallards and coots (*Fulica atra*) from a lagoon in Spain impacted by Pb shot (Martinez-Haro et al., 2011). ALAD ratio in mallards decreased linearly with blood Pb levels between 6 and 40 µg Pb/dL, and at Pb levels of <20 µg Pb/dL effects on several antioxidant enzymes were observed in coots. Although the primary route of exposure to the birds was via ingestion of Pb shot, effects were observed lower than 20 µg Pb/dL, the background level frequently applied to Pb exposures in birds (Martinez-Haro et al., 2011; Brown et al., 2006).

Consideration of toxicity of Pb to vertebrate embryos that develop surrounded by a protective egg shell has been expanded since the 2006 Pb AQCD. Pb treatment of mallard duck (*Anas platyrhynchos*), eggs by immersion in an analytically verified concentration of 100 µg Pb/L for 30 minutes on day 0 of development did not increase malformations or mortality of embryos (Kertész and Fáncsi, 2003). However, immersion of eggs in 2,900 µg Pb/L under the same experimental conditions resulted in increased rate of mortality and significant malformations including hemorrhages of the body, stunted growth, and absence of yolk sac circulatory system (Kertész et al., 2006). The second study was conducted to emulate environmental levels of Pb following a dam failure in Hungary.

6.4.6 Exposure and Response of Freshwater Species

Evidence regarding exposure-response relationships and potential thresholds for Pb effects on aquatic populations can inform determination of standard levels that are protective of aquatic ecosystems. The Annex of the 2006 Pb AQCD (U.S. EPA, 2006c) summarized data on exposure-response functions for invertebrates (Table AX7-2.4.1) and

fish (Table AX7-2.4.2). The recent exposure-response studies in this section expand on the findings from the 2006 Pb AQCD with information on newly-tested organisms (including microalgae, invertebrate, amphibian and fish species) (Table 6-5 and Figure 6-5). Overall, new data for freshwater invertebrates generally support the previous finding of sensitivity of juvenile lifestages and indicates some effects of Pb observed in some species at concentrations of Pb reported in freshwater environments. (Table 6-2). All reported values are from exposures in which concentrations of Pb were analytically verified unless nominal concentrations are stated.

The aquatic macrophyte *Lemna minor* (duckweed) exhibited a EC_{50} for growth inhibition of 6,800 µg Pb/L in a 4-day exposure and 5,500 µg Pb/L for a 7-day exposure to a range of Pb concentrations from 100 to 9,970 µg Pb/L (Dirilgen, 2011). Growth (measured as biomass) was slightly increased at 100 and 200 µg Pb/L and then decreased in subsequent concentrations. In an assay using nominal concentrations of Pb the aquatic freshwater microalgae *Scenedesmus obliquus* was significantly more sensitive to Pb exposure than *Chlorella vulgaris* algae, although these authors stated that both appeared to be very tolerant of the heavy metal. Laboratory 48-hour standard toxicity tests were performed with both of these species and respective EC_{50} values of 4,040 and 24,500 µg Pb/L for growth as measured by cell division rate were derived (Atici et al., 2008).

Exposure-response data for freshwater invertebrates provide evidence for effects of Pb at concentrations of Pb encountered in U.S. surface waters. In the 2006 Pb AQCD, effects of Pb-exposure in amphipods (*H. azteca*) and water fleas (*D. magna*) were reported at concentrations as low as 0.45 µg Pb/L. Effective concentrations for aquatic invertebrates were found to range from 0.45 to 8,000 µg Pb/L. Since the 2006 Pb AQCD, recent studies have identified the freshwater snail *L. stagnalis* as a species that is extremely sensitive to Pb exposure (Grosell and Brix, 2009; Grosell et al., 2006b). Growth of juvenile *L. stagnalis* was inhibited below the lowest concentration tested resulting in an EC_{20} of <4 µg Pb/L. In the same study, the NOEC was 12 µg Pb/L and the LOEC was 16 µg Pb/L. In contrast, freshwater juvenile ramshorn snails *M. cornuarietis* were less sensitive to Pb with the same LOEC for hatching rate and LC_{50}, calculated to be about 10,000 µg Pb/L based on nominal exposure data (Sawasdee and Köhler, 2010).

Additional studies on Pb effects in aquatic invertebrates published since the 2006 Pb AQCD provide further evidence for differences in sensitivity of different lifestages of aquatic organisms to Pb. In the freshwater mussel, *L. siliquoidea* (fatmucket) a Pb concentration response was observed in which newly transformed (5-day-old) juveniles were the most sensitive lifestage in a 96-hour toxicity test when compared to acute and chronic results with other lifestages (Wang et al., 2010f). The 96-hour EC_{50} values for the 5-day-old *L. siliquoidea* in two separate toxicity tests were 142 and

298 µg Pb/L (mean EC_{50} 220 µg Pb/L) in contrast to older juveniles (2 months old) with an EC_{50} >426 µg/L. The 24-hour median effect concentration for glochidia (larvae) of *L. siliquoidea* in 48-hour acute toxicity tests was >299 µg/L. A 28-day exposure chronic value of 10 µg Pb/L was obtained from 2-month-old *L. siliquoidea* juveniles, and was the lowest genus mean chronic value ever reported for Pb (Wang et al., 2010f). A 96-hour test on newly transformed juveniles was also conducted on *Lampsilis rafinesqueana* (Neosho mucket), a mussel that is a candidate for the endangered species list. The EC_{50} for this species was 188 µg Pb/L.

Different lifestages of chironomids have been shown to have varying sensitivity to Pb exposure in several studies available since the 2006 Pb AQCD. The acute toxicity of Pb to first-instar *C. riparius* larvae was tested in soft water, with hardness of 8 mg/L as $CaCO_3$ (Béchard et al., 2008). The 24-hour LC_{50} of 610 µg Pb/L for first instar *C. riparius* larvae was much lower than previous values reported for later instars in harder water. In a chronic test with *Chironomus tentans*, (8 day-old larvae exposed to Pb until emergence [approximately 27 days]), the NOEC was 109, and the LOEC was 497 µg Pb/L (Grosell et al., 2006b). The EC_{20} for reduced growth and emergence of the midge *Chironomus dilutus* was 28 µg Pb/L, observed in a 55-day exposure, while the same species had a 96-hour LC_{50} of 3,323 µg Pb/L (Mebane et al., 2008). In fourth instars of the freshwater midge larvae *Chironomus javanus* the 24, 48, 72 and 96 hour LC_{50} values were 20,490, 6,530, 1,690 and 720 µg Pb/L, respectively (Shuhaimi-Othman et al., 2011c). This was comparable to the 96-hour LC_{50} (400 µg Pb/L) in the midge larvae *Culicoides furens* (Vedamanikam and Shazilli, 2008a). In the same study, the 96-hour LC_{50} for *Chironomus plumosus* ranged from 8,300 µg Pb/L to 16,210 µg Pb/L under different temperatures indicating the role of environmental factors in modulation of toxicity and differences in sensitivity to Pb even among related species.

Cladocerans are commonly tested aquatic organisms, with data from three species: *D. magna, D. pulex* and *Ceriodaphnia dubia*, representing approximately 70% of available metal toxicological literature on this group (Wong et al., 2009). Recent studies have been conducted with *C. dubia* and acute toxicity values for other cladocerans as well as sublethal endpoints for *D. magna* are available. In a series of 48 hour acute toxicity tests with *C. dubia* conducted in a variety of natural waters across North America, LC_{50} values ranged from 29 to 1,180 µg Pb/L and were correlated with DOC (Esbaugh et al., 2011). Median lethal concentrations for *Moina micrura* (LC_{50} 690 µg Pb/L), *Diaphanosoma birgei* (LC_{50} 3,160 µg Pb/L), and *Alona rectangular* (LC_{50} 7,060 µg Pb/L) indicate differences in sensitivity to Pb in these freshwater cladocerans from Mexico (García-García et al., 2006). Several additional studies available since the 2006 Pb AQCD report exposure response values for Daphnia that are based an nominal data: an acute study of Pb with *D. pulex* identified a 48-hour LC_{50} of 4,000 µg/L for this

species (Theegala et al., 2007) and the EC_{50} for swimming inhibition in neonate *D. magna* exposed to Pb nitrate for 24 hours was 18,153 µg Pb/L (Ha and Choi, 2009).

Rotifers are among the most sensitive aquatic genera to Pb with wide variation in LC_{50} values reported between species (Pérez-Legaspi and Rico-Martínez, 2001). For example, in the rotifer genus *Lecane*, a 22-fold difference in LC_{50} values was observed in 48-hour exposure to Pb between *L. hamata, L. luna* and *L. quadridentata*. (Pérez-Legaspi and Rico-Martínez, 2001). *L. luna* was most sensitive to Pb toxicity with a 48-hour LC_{50} of 140 µg Pb/L. In a 48-hour toxicity test with the rotifer *Brachionus calyciflorus*, an NOEC (194 µg Pb/L), an LOEC (284 µg Pb/L), and an EC_{20} of 125 µg Pb/L was established for this species (Grosell et al., 2006b). The freshwater rotifer *Euchlanis dilatata* 48 hour LC_{50} was 35 µg Pb/L using neonates hatched from asexual eggs (Arias-Almeida and Rico-Martínez, 2011). In this study the authors estimated the NOEC to be 0.1 µg Pb/L and the LOEC to be 0.5 µg Pb/L. In contrast, for rotifer *Brachionus patulus* neonates, the 24-hour LC_{50} was 6,150 µg Pb/L, however, this value was based on nominal exposures (García-García et al., 2007).

Exposure-response assays on other freshwater species have been conducted since the 2006 Pb AQCD. The 24-hour LC_{50} for larvae of *C. quinquefasciatus* mosquitoes was 180 µg Pb/L (Kitvatanachai et al., 2005). A 48-hour LC_{50} of 5,200 µg Pb/L was observed in water-only exposures of the blackworm *Lumbriculus variegatus (*Penttinen et al., 2008*)*. In the mayfly *Baetis tricaudatus*, the 96-hour LC_{50} was 664 µg Pb/L (Mebane et al., 2008). An EC_{20} value of 66 µg Pb/L was derived for *B. tricaudatus* by quantifying the reduction in the number of molts over a 10-day exposure to Pb (Mebane et al., 2008). The number of molts was significantly less than the control (average of 14 molts over 10 days) at concentrations of 160 µg Pb/L and higher with the lowest number of molts (average of 5.3 molts over 10 days) observed in the highest concentration (546 µg Pb/L). In the freshwater ostracod *Stenocypris major*, the 96-hour LC_{50} was 526 µg Pb/L (Shuhaimi-Othman et al., 2011b). In another freshwater crustacean, the prawn *Macrobrachium lancesteri*, the 96-hour LC_{50} was 35 µg Pb/L in soft water (<75 mg/L as $CaCO_3$) (Shuhaimi-Othman et al., 2011a).

In the studies reviewed for the 2006 Pb AQCD, freshwater fish demonstrated adverse effects at concentrations ranging from 10 to >5,400 µg Pb/L, generally depending on water quality parameters (e.g., pH, hardness, salinity) (U.S. EPA, 2006c). Pb tended to be more toxic in longer-term exposures and correlated to Pb-uptake in tissues. Table AX7-2.4.2 of the 2006 Pb AQCD summarizes effects of Pb to fish. A series of studies published since the 2006 Pb AQCD have been conducted and have further elucidated the influence of water chemistry parameters on Pb uptake and toxicity in fathead minnow resulting in additional dose-response data for this species. Grosell et al. (2006b)

conducted a series of 30-day exposures with larval fathead minnow in which varying concentrations of Ca^{2+} (as $CaSO_4$) and DOC were tested. The effects of reduced pH (6.7) and increased pH (8.1) compared to a control pH of 7.4 on Pb toxicity were also assessed in this study. DOC, $CaSO_4$ and pH influenced Pb toxicity considerably over the range of water parameters tested. For example, The 30-day LC_{50} for low hardness (19 mg $CaCO_3$/L) in basic test water (base water) was 39 µg dissolved Pb/L and the highest LC_{50} value (obtained from the protection from increased concentrations of DOC and $CaSO_4$) was 1,903 µg dissolved Pb/L (Grosell et al., 2006a). This range in LC_{50} values for larval fathead minnows for differing concentrations of DOC and hardness clearly demonstrates the importance of the chemistry of the exposure medium to Pb toxicity.

Mager et al. (2010) conducted 300-day chronic toxicity tests at 35 and 120 µg Pb/L with fathead minnow under conditions of varied DOC and alkalinity to assess the effects of these water quality parameters on fish growth and Pb-uptake. In additional tests with fathead minnow, Mager et al. (2011b) conducted both 96-hour acute and 30-day chronic tests to further characterize Ca^{2+}, DOC, pH, and alkalinity values on Pb toxicity. Increased Ca^{2+}, DOC and $NaHCO_3$ concentration afforded protection to minnows in acute studies. The role of pH in Pb toxicity is complex and likely involves Pb speciation and competitive interaction of H^+ with Pb^{2+} (Mager et al., 2011b). In a series of 96-hour acute toxicity tests with fathead minnow conducted in a variety of natural waters across North America, LC_{50} values ranged from 41 to 3,598 µg Pb/L and no Pb toxicity occurred in three highly alkaline waters (Esbaugh et al., 2011).

In the 2006 Pb AQCD, fish lifestage was recognized as an important variable in determining the sensitivity of these organisms to Pb. Recent data available since the 2006 Pb AQCD (U.S. EPA, 2006c) support the findings of increased sensitivity of juvenile fish to Pb when compared to adults. Acute (96-hour) and chronic (60-day) early-lifestage test exposures were conducted with rainbow trout to develop acute-chronic ratios (ACR's) for this species (Mebane et al., 2008). Two early-lifestage chronic tests were conducted, the first with an exposure range of 12-384 µg Pb/L (69 days) at 20 mg $CaCO_3$/L water hardness and the second with an exposure range of 8 to 124 µg Pb/L (62 days) and a water hardness of 29 mg $CaCO_3$/L. In the 69-day test, the following chronic values were observed for survival: NOEC=24 µg Pb/L, maximum acceptable toxicant concentration (MATC)=36 µg Pb/L, EC_{10}=26 µg Pb/L, EC_{20}=34 µg Pb/L, and LC_{50}=55 µg Pb/L. Results from the 62-day test, with fish length as the endpoint, were NOEC=8 µg Pb/L, MATC=12 µg Pb/L, EC_{10}=7µg Pb/L, EC_{20}=102 µg Pb/L and LC_{50}=120 µg Pb/L. In acute tests with rainbow trout run concurrently with the chronic tests, 96-hour LC_{50} values were 120 and 150 µg Pb/L, respectively. Data from this study resulted in ACR's for trout lower than previously reported. The low ACR values were due to the acute tests which produced LC_{50} values that were 10 to 25 times lower than

earlier studies with trout (Mebane et al., 2008). The authors speculated that the lower LC_{50} values were due to the age of the fish used in the study (two to four week old fry) and that testing with larger and older fish may not be protective of more sensitive lifestages.

There have been only a few recent exposure-response studies in amphibians since the 2006 Pb AQCD. Southern leopard frog tadpoles exposed to Pb in sediment (45 to 7,580 mg Pb/kg dry weight) with corresponding sediment pore water concentrations from 123 to 24,427 µg Pb/L from embryonic stage to metamorphosis exhibited concentration-dependent effects on survival (Sparling et al., 2006). The LC_{50} value for Pb in sediment was 3,738 mg Pb/kg, which corresponds to 12,539 µg Pb/L in sediment pore water. In the same study, concentration-dependent effects on skeletal development were observed. The 40 day-EC_{50} for deformed spinal columns in the tadpoles was 1,958 mg Pb/kg (corresponding to 6,734 µg Pb/L sediment pore water) and the 60 day-EC_{50} was 579 mg Pb/kg (corresponding to 1,968 µg Pb/L sediment pore water) (Sparling et al., 2006).

6.4.7 Freshwater Community and Ecosystem Effects

As discussed in the 1986 Pb AQCD (U.S. EPA, 1986b) and the 2006 Pb AQCD (U.S. EPA, 2006b), exposure to Pb is likely to have impacts in aquatic environments via effects at several levels of ecological organization (organisms, populations, communities, or ecosystems). These effects resulting from toxicity of Pb would be evidenced by changes in species composition and richness, in ecosystem function, and in energy flow. The 2006 Pb AQCD concluded that, in general, there was insufficient information available for single materials in controlled studies to permit evaluation of specific impacts on higher levels of organization (beyond the organism). Furthermore, Pb rarely occurs as a sole contaminant in natural systems making the effects of Pb difficult to ascertain. New information on effects of Pb at the population, community, and ecosystem level is reviewed below.

In laboratory studies reviewed in the 2006 Pb AQCD and in more recent studies, Pb exposure has been demonstrated to alter predator-prey interactions, as well as feeding and avoidance behaviors. In aquatic ecosystems there are field studies reviewed in the 1977 Pb AQCD (U.S. EPA, 1977), the 1986 Pb AQCD (U.S. EPA, 1986b), the 2006 Pb AQCD (U.S. EPA, 2006b) and more recent studies that report reductions of species abundance, richness or diversity particularly in benthic macroinvertebrate communities coexisting with other metals where the sources of Pb were from mining or urban effluents. Additionally, field studies have linked Pb contamination to reduced primary productivity and respiration, and to altered energy flow and nutrient cycling.

However, because of the complexity inherent in defining such effects, there are relatively few available population, community, or ecosystem level studies that conclusively relate Pb exposure to aquatic ecosystem effects. In addition, most of the available work is related to point-source Pb contamination, with very few studies considering the effects of diffuse Pb pollution. Both plant species and habitat type were determined to be factors affecting the rate of Pb accumulation from contaminated sediments. While the rooted aquatic plant *E. canadensis* was observed to accumulate the highest concentrations of Pb, the authors concluded that submerged macrophytes (versus emergent plants) as a group were the most likely to accumulate Pb and other heavy metals (Kurilenko and Osmolovskaya, 2006). This would suggest that certain types of aquatic plants, such as rooted and submerged species, may be more susceptible to aerially-deposited Pb contamination, resulting in shifts in plant community composition as a result of Pb pollution.

Alteration of macrophyte community composition was demonstrated in the presence of elevated surface water Pb concentrations at three lake sites impacted by mining effluents (Mishra et al., 2008). A total of 11 species of macrophytes were collected. Two sites located 500 meters and 1,500 meters downstream from the mine discharge point (study sites 2 and 3) exhibited similar dissolved Pb concentrations (78 to 92 µg Pb/L, depending on season) and contained six and eight unique macrophyte species, respectively. The site nearest the discharge point of the mine effluent (study site 1) had the highest Pb concentrations (103 to 118 µg Pb/L) and the lowest number of resident macrophyte species; these included *E. crassipes*, *L. minor*, *Azolla pinnata* and *S. polyrrhiza*. Based on analysis of plant tissue Pb concentrations, the authors theorized that certain species may be more able to develop Pb tolerant eco-types that can survive at higher Pb concentrations (Mishra et al., 2008). In field studies available for certain freshwater habitats, exposure to Pb has been shown to result in significant alterations of invertebrate communities. Macroinvertebrate community structure in mine-influenced streams was determined to be significantly correlated to Pb sediment pore water concentrations. Multiple invertebrate community indices, including Ephemeroptera, Plecoptera, Trichoptera (EPT) taxa richness, Missouri biotic index, and Shannon-Wiener diversity index, were integrated into a macroinvertebrate biotic condition score (Poulton et al., 2010). These scores were determined to be significantly lower at sample sites downstream from mining sites where Pb pore water and bulk sediment concentrations were elevated. Sediment Pb, Cd, and Zn levels were inversely correlated to mussel taxa richness in the Spring River basin encompassing sites in Kansas, Missouri and Oklahoma overlapping a former Pb and Zn mining and processing area (Angelo et al., 2007). In sites upstream of the mining area, 21 to 25 species of mussels were present whereas in sites downstream, only 6 to 8 species were observed.

Rhea et al. (2006) examined the effects of multiple heavy metals in the Boulder River, Montana, watershed biofilm on resident macroinvertebrate assemblages and community structure, and determined that, among all the metals, biofilm Pb concentrations exerted the greatest influence on the macroinvertebrate community indices. Pb biofilm concentrations were significantly correlated with reduced EPT taxa richness, reduced EPT abundance, and an increase in Diptera species abundance. Interestingly, Pb concentrations in invertebrate tissues were correlated to an increase in Hydropsychidae caddisfly abundance, but this may have resulted from the intrinsically high variability in tissue Pb concentrations. The authors concluded that Pb-containing biofilm represented a significant dietary exposure for impacted macroinvertebrate species, thus altering invertebrate community metrics (Rhea et al., 2006).

Kominkova and Nabelkova (2005) examined ecological risks associated with metal contamination (including Pb) in small urban streams. Although surface water Pb concentrations in monitored streams were determined to be very low, concentrations of the metal in sediment were high enough to pose a risk to the benthic community (e.g., 34 to 101 mg Pb/kg). These risks were observed to be linked to benthic invertebrate functional feeding group, with collector-gatherer species exhibiting larger body burdens of heavy metals than other groups (Kominkova and Nabelkova, 2005). In contrast, benthic predators and collector-filterers accumulated significantly lower metals concentrations. Consequently, it is likely that sediment-bound Pb contamination would differentially affect members of the benthic invertebrate community, potentially altering ecosystem dynamics.

Invertebrate functional feeding group may also affect invertebrate Pb body burdens in those systems where Pb bioconcentration occurs. The predaceous zooplanktonic rotifer, *A. brightwellii* collected from a Pb-impacted freshwater reservoir in Mexico, contained 384 ng Pb/mg and exhibited a water-to-tissue BCF of 49,344. The authors theorized that Pb biomagnification may have been observed in this case because the cladoceran *M. micrura* is both a known Pb accumulator and a favorite prey item of the rotifer (Rubio-Franchini et al., 2008). They showed that *M. micrura* had twice the Pb body burden of *D. similis*, another grazing cladoceran species present in the reservoir. These two species exhibited average Pb tissue concentrations of 57 and 98 ng Pb/mg, respectively, with respective water column BCFs of 9,022 and 8,046. Conversely, an examination of the simultaneous uptake of dissolved Pb by the algae *P. subcapitata* and the cladoceran *D. magna* suggests that the dietary exposure route for the water column filter-feeder is minor. Although Pb accumulated in the algal food source, uptake directly from the water column was determined to be the primary route of exposure for *D. magna* (Komjarova and Blust, 2009c).

For many invertebrate species, sediment Pb concentrations may be the most important driver in determining Pb uptake. For instance, while Hg and Cd body burdens in lentic invertebrates were affected by lake ecological processes (e.g., eutrophication), a similar effect was not observed for Pb concentrations in crayfish tissue, despite a high variability between sites (Larsson et al., 2007). Although this may be a result of differing bioaccumulation tendencies, the authors suggested that other factors, including the potential for sediment exposures, may be responsible for Pb uptake in lentic invertebrates.

A field survey of fishes in the Viburnum Trend Pb-Zn mining district in southeast Missouri available since the 2006 Pb AQCD, found that species richness and species density of riffle-swelling benthic fishes were negatively correlated with metal concentrations in pore water and in fish in mining impacted streams (Allert et al., 2009b). Density of Ozark sculpin (*Cottus hypselurus*) and banded sculpin (*Cottus carolinae*) were positively correlated with distance from mining sources.

In addition to the ecological effects discussed above, there is additional evidence that Pb exposure could alter bacterial infection (and potentially disease transmission) in certain fish species. Following 96-hour exposures to 4,000 µg Pb/L, bacterial density in *Channa punctatus* fish was observed to be significantly altered when compared to non-exposed fish. Bacteria population densities in fish spleen, gills, liver, kidneys and muscle tissues were higher following Pb exposure, with bacterial abundance in the gills too numerous to quantify (Pathak and Gopal, 2009). In addition, bacteria inhabiting Pb-exposed fish were more likely to exhibit antibacterial resistance than colonies isolated from non-exposed fish. Although the mechanism remains unknown, this study suggests that Pb exposure may increase the likelihood of infection in fish, potentially affecting fish abundance and recruitment.

In summary, despite the fact that alterations of macrophyte communities may be highly visible effects of increased sediment Pb concentrations, several recently published papers propose that ecological impacts on invertebrate communities are also significant, and can occur at environmental Pb concentrations lower than those required to impact plant communities. High sediment Pb concentrations were linked to shifts in amphipod communities inhabiting plant structures, and potentially to alterations in ecosystem nutrient processing through selective pressures on certain invertebrate functional feeding groups (e.g., greater bioaccumulation and toxic effects in collector-gatherers versus predators or filter-feeders). Increased sediment pore water Pb concentrations were demonstrated to likely be of greater importance to invertebrate communities, as well. Interestingly, recent research also suggests that Pb exposure can alter bacterial

infestations in fish, increasing both microbial density and resilience, and potentially increasing the likelihood of serious disease outbreak.

6.4.8 Critical Loads in Freshwater Systems

The general concept and definition of critical loads is introduced in Section 6.1.3 of this chapter [also see Section 7.3 of the 2006 Pb AQCD (U.S. EPA, 2006c)]. Critical load values are linked to critical limits of Pb for endpoints/receptors of interest in the ecosystems, such as blood Pb. Some important critical limits for Pb in aquatic ecosystems are discussed in this section along with information on aquatic critical loads for Pb.

Unit World Models (UWM) have been used to calculate critical loads for metals in aquatic ecosystems. These models couple an ecotoxicity model, the BLM, to a speciation/complexation model, the Windermere Humic Adsorption Model (WHAM), then to the multi-species fate model, TRANsport-SPECiation (TRANSPEC). Gandhi et al. (2011) applied a UWM approach to estimate speciation/complexation, fate and critical loads using lakes of three different trophic status. A high percentage of colloidal-bound Pb was found in the eutrophic and mesotrophic lakes (75-80%) versus the oligotrophic lakes (2%), owing to the high affinity of Pb to DOM. Pb concentrations were lowest for mesotrophic and highest for oligotrophic systems. Critical loads were not calculated for Pb; however, for the other metals tested the critical load was lowest in the oligotrophic and highest in the eutrophic systems.

A critical load of 39.0 g Pb/m^2 per year (0.19 mol Pb/ m^2 per year) was calculated for a generalized lake in the Sudbury area of the Canadian Shield using TICKET-UWM based on acute toxicity data for *D. magna*. (Farley et al., 2011). The model was set up to calculate critical loads of metals by specifying free metal ion activity or the critical biotic ligand concentration. This critical load for Pb was much higher than for Cu, Ni and Zn and the authors attribute this difference to the strong binding of Pb to particulate organic matter and the sequestration of $PbCO_3$ in sediment.

Given the heterogeneity of ecosystems exposed to Pb, and the differences in expectations for ecosystem services attached to different land uses, it is expected that there will be a range of critical load values for Pb for soils and waters within the U.S. Refer to Section 7.3.6 of the 2006 Pb AQCD for additional discussion of critical loads of Pb in aquatic systems.

6.4.9 Characterization of Sensitivity and Vulnerability in Freshwater Systems

Data from the literature indicate that exposure to Pb may affect survival, reproduction, growth, metabolism, and development in a wide range of freshwater aquatic species. Often, species differences in metabolism, sequestration, and elimination rates control relative sensitivity and vulnerability of exposed organisms. Diet and lifestage at the time of exposure also contribute significantly to the determination of sensitive and vulnerable populations and communities. Further, environmental conditions in addition to those discussed as affecting bioavailability (Sections 6.4.3 and 6.4.4) may also alter Pb toxicity. The 2006 Pb AQCD (U.S. EPA, 2006b) reviewed the effects of genetics, age, and body size on Pb toxicity. While genetics appears to be a significant determinant of Pb sensitivity, effects of age and body size are complicated by environmental factors that alter metabolic rates of aquatic organisms. A review of the more recent literature corroborated these findings, and identified seasonally-affected physiological changes and lifestage as other important determinants of differential sensitivity to Pb.

6.4.9.1 Seasonally-Affected Physiological Changes

A study by Duman et al. (2006) identified species and seasonal effects of Pb uptake in aquatic plants. *P. australis* accumulated higher root Pb concentrations than *S. lacustris*. Additionally, the *P. australis* Pb accumulation factor was significantly higher during the winter versus other seasons, while the Pb accumulation factor for *S. lacustris* was greatest in spring and autumn. The Pb accumulation factor for a third species, *P. lucens*, was greatest in autumn (Duman et al., 2006). Most significantly, these changes in bioaccumulation were not linked with biomass increases, indicating that species-dependent seasonal physiological changes may control Pb uptake in aquatic macrophytes (Duman et al., 2007). Significant interspecies differences in Pb uptake were observed for plants representing the same genus (Sargassum), indicating that uptake of Pb by aquatic plants also may be governed by highly species-dependent factors (Jothinayagi and Anbazhagan, 2009).

Heier et al. (2009) established the speciation of Pb in water draining from a shooting range in Norway and looked at the time-dependent accumulation in brown trout. They found that elevated concentrations of Pb, particularly high molecular weight (>10 kDa) positively charged Pb species associated with organic matter, were correlated with high flow episodes and accumulation of Pb on gills and in the liver. Thus, high flow episodes can remobilize metals from a catchment and induce stress to aquatic organisms.

6.4.9.2 Increased Nutrient Uptake

Singh et al. (2010) proposed that metal-resistant plants have the capacity to not only up-regulate antioxidant synthesis, but also have the ability to increase nutrient consumption and uptake to support metal sequestration and detoxification via production of antioxidants (Singh et al., 2010). Therefore, it is likely that such plant species would be significantly less susceptible to Pb exposure than those species without those abilities.

6.4.9.3 Temperature and pH

Water temperature also appears to affect the toxicity of Pb to aquatic organisms, with higher temperatures leading to greater responses. Pb toxicity to crayfish increased 7 to 10% when the water temperature was increased by 4 °C, and by 14% when the temperature increased by 7 °C. The authors determined that the increased toxicity was a result of the negative impact of Pb on crayfish respiration, which was exacerbated by the lower dissolved oxygen concentrations at higher water temperatures (Khan et al., 2006). In a study of the combined effects of temperature and Pb concentration on two freshwater rotifer species, *Brachionus havanaensis* and *B. rubens*, population growth was measured in three nominal concentrations of Pb as Pb chloride (50, 100 and 200 µg Pb/L) for 15 days at either 22 °C or 32 °C (Montufar-Melendez et al., 2007). At 22 °C, population growth of *B. havanaensis* was suppressed by *B. rubens* regardless of Pb treatment. At the higher temperature, there was no population increase of *B. rubens* at any Pb concentration. In the controls, population growth rates of *B. havanaensis*, but not *B. rubens*, increased with an increase in temperature. These studies highlight the role of temperature in Pb toxicity in organisms adapted to low temperatures.

The sequestration ability of *L. minor* macrophytes was similarly impacted by increased surface water temperature; plants absorbed a maximum Pb concentration of 8.6 mg /g at 30 °C, while uptake at 15 °C was only 0.3 mg/g (Uysal and Taner, 2009). Decreased pH was also demonstrated to increase the uptake of environmental Pb in aquatic plants (Wang et al., 2010b; Uysal and Taner, 2009). Lower pH was shown to result in increased sensitivity to Pb in juvenile fathead minnows in 30-day exposure to Pb of varying concentrations (Grosell et al., 2006a). Additionally, Birceanu et al. (2008) determined that fish (specifically rainbow trout) were more susceptible to Pb toxicity in acidic, soft waters characteristic of sensitive regions in Canada and Scandinavia. Hence, fish species endemic to such systems may be more at risk from Pb contamination than fish species in other habitats.

6.4.9.4 Lifestage

It is clear that certain stages of a life cycle are more vulnerable to Pb. A comparison of *C. riparius* Pb LC_{50} values derived from toxicity tests with different instars indicates a significant effect of lifestage on Pb sensitivity for aquatic invertebrates. Béchard et al. (2008) calculated a first instar *C. riparius* 24-hour LC_{50} value of 613 µg Pb/L, and contrasted this value with the 24-hour and 48-hour LC_{50} values derived using later instar larvae—350,000 and 200,000 µg Pb/L, respectively. This disparity would suggest that seasonal co-occurrence of aquatic Pb contamination and sensitive early instars could have significant population-level impacts (Béchard et al., 2008). Similarly, Wang et al. (2010f) demonstrated that the newly transformed juvenile mussels, *L. siliquoidea* and *L. rafinesqueana*, at 5 days old were more sensitive to Pb exposure than were glochidia or two to six month- old juveniles, suggesting that Pb exposure at particularly sensitive lifestages could have a significant influence on population viability (Wang et al., 2010f).

Evidence for differences in susceptibility to Pb at distinct lifestages is also available for freshwater fish. In chronic (60-day) early-lifestage test exposures conducted with rainbow trout to develop ACR's for this species the study resulted in ACR's for rainbow trout lower than previously reported due to the acute tests which produced LC_{50} values that were 10 to 25 times lower than earlier studies with trout (Mebane et al., 2008). The authors speculated that the lower LC_{50} values were due to the age of the fish used in the study (two to four week old fry) and that testing with larger and older fish may not be protective of more sensitive lifestages. Post-hatching stages of the African catfish were more sensitive than the embryonic stage to Pb-exposure and the authors attributed this apparent protective effect to the presence of a hardened chorion in embryos (Osman et al., 2007a).

6.4.9.5 Species Sensitivity

Species-specific Ca^{2+} requirements have been shown to affect the vulnerability of aquatic organisms to Pb. The snail, *L. stagnalis*, exhibits an unusually high Ca^{2+} demand due to $CaCO_3$ formation required for shell production and growth, and exposure to Pb prevents the uptake of needed Ca^{2+}, leading to toxicity. Consequently, aquatic species that require high assimilation rates of environmental Ca^{2+} for homeostasis are likely to be more sensitive to Pb contamination (Grosell and Brix, 2009). Grosell and colleagues also noted that reduced snail growth following chronic Pb exposure was likely a result of reduced Ca^{2+} uptake (Grosell et al., 2006b).

There is some indication that molting may comprise an additional sequestration and excretion pathway for aquatic animals exposed to Pb (Soto-Jiménez et al., 2011a; Mohapatra et al., 2009; Tollett et al., 2009; Bergey and Weis, 2007). Libellulidae dragonfly nymphs (Tollett et al., 2009) have been shown to preferentially sequester Pb in exoskeleton tissue. Consequently, aquatic arthropod species and those species that shed their exoskeleton more frequently may be able to tolerate higher environmental Pb concentrations than non-arthropods or slow-growing molting species, as this pathway allows them to effectively lower Pb body burdens.

In contrast, the effect of Pb exposure on fish bacterial loads demonstrated by Pathak and Gopal (2009) suggest that infected fish populations may be more at risk to the toxic effects of Pb than healthier species. Aqueous Pb was demonstrated to both increase bacteria density in several fish organs and to improve the likelihood of antibacterial resistance (Pathak and Gopal, 2009).

Tolerance to prolonged Pb exposure may develop in aquatic invertebrates and fish. Multi-generational exposure Pb appears to confer some degree of metal tolerance in invertebrates such as *C. plumosus* larvae; consequently, previous population Pb exposures may decrease species' susceptibility to Pb contamination (Vedamanikam and Shazilli, 2008b). However, the authors noted that metal tolerant larvae were significantly smaller than larvae reared under clean conditions, and that transference of Pb-tolerant *C. plumosus* larvae to clean systems resulted in a subsequent loss of tolerance. Evidence of acclimation to elevated Pb in fathead minnow was suggested in the variations in ionoregulatory parameters that were measured on day 10 and 30 in fish exposed to 115 µg Pb/L for 30 days. At the end of the experiment, whole body Ca^{2+} was elevated while Na^{+} and K^{+} recovered from elevated levels at 30 days (Grosell et al., 2006a).

A series of species sensitivity distributions constructed by Brix et al. (2005) in freshwater systems indicated that sensitivity to Pb was greatest in crustacean species, followed by coldwater fish, and warmwater fish and aquatic insects, which exhibited a similar sensitivity. Further, analysis of both acute and chronic mesocosm data sets indicated that Pb-contaminated systems exhibited diminished species diversity and taxa richness following both types of exposure (Brix et al., 2005). Wong et al. (2009) constructed Pb species sensitivity distributions for both cladoceran and copepod freshwater species. A comparison of the two curves indicated that cladoceran species, as a group, were more sensitive to the toxic effects of Pb than were copepods, with respective hazardous concentration values for 5% of the species (HC5) values of 35 and 77 µg Pb/L. This difference in sensitivities would indicate that cladoceran species are more likely to be impacted at lower environmental Pb concentrations than copepods, potentially altering community structures or ecosystem functions (Wong et al., 2009).

6.4.9.6 Ecosystem Vulnerability

Relative vulnerability of different freshwater ecosystems to effects of Pb can be inferred from the information discussed above on species sensitivity and the influence of water quality variables on the bioavailability and toxicity of Pb. It is, however, difficult to categorically state that certain freshwater plant, invertebrate or vertebrate communities are more vulnerable to Pb than others, since toxicity is dependent on many variables and data from field studies are complicated by co-occurrence of other metals and alterations of pH, such as in mining areas. Aquatic ecosystems with low pH and low DOM are likely to be the most sensitive to the effects of atmospherically-deposited Pb. Examples of such systems are acidic, soft waters such as sensitive regions in Canada and Scandinavia (Birceanu et al., 2008). In the U.S., aquatic systems that may be more sensitive to effects of Pb include habitats that are acidified due to atmospheric deposition of pollutants, runoff from mining activities or lakes and streams with naturally occurring organic acids. Hence, fish and invertebrate species endemic to such systems may be more at risk from Pb contamination than corresponding species in other habitats.

6.4.10 Ecosystem Services Associated with Freshwater Systems

Pb deposited on the surface of, or taken up by organisms has the potential to alter the services provided by freshwater biota to humans although the directionality of impacts is not always clear. For example, aquatic macrophytes provide a service by sequestering Pb. At the same time, the uptake of Pb by plants may result in toxicological effects associated with Pb exposure and decreased capacity of wetland species to remove contaminants. At this time, few publications address Pb impacts on ecosystem services associated with freshwater systems and most studies focus on wetlands rather than lakes and streams. Pb can affect the ecological effects in each of the four main categories of ecosystem services (Section 6.1.2) as defined by Hassan et al. (2005). These effects are sorted into ecosystem services categories and summarized here:

- Supporting: food for higher trophic levels, biodiversity
- Provisioning: clean drinking water, contamination of food by heavy metals, decline in health of fish and other aquatic species
- Regulating: water quality
- Cultural: ecosystem and cultural heritage values related to ecosystem integrity and biodiversity, wildlife and bird watching, fishing

Freshwater wetlands are sinks for atmospheric Pb as well as Pb from terrestrial runoff (Landre et al., 2010; Watmough and Dillon, 2007). Several studies have addressed the response of natural wetlands to Pb (Odum, 2000; Gambrell, 1994). Recent reviews of

pollution control (Mander and Mitsch, 2009) or removal of metals (Marchand et al., 2010) by constructed wetlands and phytoremediation of metals by wetland plants (Rai, 2008) indicate that these systems can remove Pb from the aquatic environment and are important for water quality, sediment stabilization, nutrient cycling and shelter for aquatic biota. The use of plants as a tool for immobilization of Pb and other metals from the environment is not limited to wetland species. Recent advances in the phytoremediation of metals are reviewed in Dickinson et al. (2009). The impact of Pb on ecological services provided by specific components of aquatic systems has been considered in a limited number of studies. For example, Theegala et al. (2007) discuss the high uptake rate of Pb by *D. pulex* as the basis for a possible Daphnia-based remediation for aquatic systems.

6.4.11 Synthesis of New Evidence for Pb Effects in Freshwater Ecosystems

This synthesis of the effects of Pb on freshwater ecosystems covers information from the publication of the 2006 Pb AQCD to present. It is followed in Section 6.4.12 by determinations of causality that take into account evidence dating back to the 1977 Pb AQCD.

Evidence assessed in the present document supports the findings of the previous Pb AQCDs that waterborne Pb is highly toxic to freshwater organisms, with toxicity varying with species and lifestage, duration of exposure, form of Pb, and water quality characteristics. The studies that are available for freshwater plants, invertebrates and vertebrates include studies where Pb concentration was analytically verified and those that reported nominal concentrations (Table 6-5). Many of the studies that report nominal concentrations in media are uptake studies that subsequently quantify Pb in tissues, however, measurement of Pb in water or sediment at the beginning of an exposure is desirable when comparing laboratory studies to concentrations of Pb in freshwater systems. As reported in Section 6.2.3 and Table 6-2, the median and range of Pb concentrations in surface waters (median 0.50 µg Pb/L, range 0.04 to 30 µg Pb/L) and sediments (median 28 mg Pb/kg dry weight, range 0.5 to 12,000 mg Pb/kg dry weight) in the U.S. based on a synthesis of NAWQA data was reported in the previous 2006 Pb AQCD (U.S. EPA, 2006c).

Recent studies provide further evidence for the role of modifying factors such as pH, DOC and hardness on the effects of Pb on plants, invertebrates and vertebrates. The same Pb concentration added to water or sediment produces far greater effects under some conditions, than others. Many studies reviewed in the ISA included concentrations that

were higher than Pb found near contaminated areas. However, when multiple concentrations were used, effects gradually increased with increasing Pb exposure. Effects at lower concentrations can be implied from many studies since an exposure-response relationship to Pb was observed, although uncertainty remains in relating these findings to reported concentrations of Pb in freshwater. Many studies only report an LC_{50} value when an LC_{10} would be more relevant for consideration of effects on organisms since an effect occurring at the LC_{50} would most likely not maintain a stable population. Most available studies only report acute toxicity and are conducted at higher concentrations of Pb than found in sampling from U.S. surface waters (Table 6-2), however, exposure to Pb in freshwater systems is most likely characterized as a chronic low dose exposure.

Plants

Most recent studies on effects of Pb in freshwater algal species reviewed in Section 6.4.5.1 were conducted with nominal media exposures and effect concentrations greatly exceeded Pb reported in surface water. In studies where Pb was quantified, effect concentrations for growth (EC_{50}) for aquatic macrophytes were much higher than currently reported ambient Pb, however, some sublethal endpoints such as effects on chlorophyll were observed at lower concentrations. For example, chlorophyll a content was significantly inhibited at 210 µg Pb/L and higher in *W. arrhiza* (Piotrowska et al., 2010). An increase in biomass was reported in *L. minor* exposed to 100 or 200 µg Pb/L with inhibition observed at higher concentrations (Dirilgen, 2011). There were also numerous studies conducted at nominal Pb concentration that report effects on enzyme activities and protein content in freshwater aquatic plant species. Exposure-response relationships in which increasing concentrations of Pb lead to increasing effects were consistently observed for freshwater aquatic plants.

Recent studies on bioavailability of Pb in aquatic plants and algae support the findings of previous Pb AQCDs that plants tend to sequester larger amounts of Pb in their roots than in their shoots and provide additional evidence for species differences in compartmentalization of sequestered Pb and responses to Pb in water and sediments. Given that atmospherically-derived Pb is likely to become sequestered in sediments, uptake by aquatic plants is a significant route of Pb removal from sediments, and a potential route for Pb mobilization into the aquatic food web. Although there are some similarities to Pb accumulation observed in terrestrial plants (e.g., preferential sequestration of the metal in root tissue), Pb appears to be more bioavailable in sediment than it is in soil.

Invertebrates

The largest body of evidence for effects of Pb at or near concentrations of this metal found in surveys of surface waters of the U.S. is for invertebrates and recent studies reviewed in Sections 6.4.5.2 and 6.4.6 further support this observation. Exposure-response relationships in which increasing concentrations of Pb lead to increasing effects were consistently observed for freshwater invertebrates. Among the most sensitive species, growth of juvenile freshwater snails *L. stagnalis* was inhibited at an EC_{20} of <4 µg Pb/L. (Grosell and Brix, 2009; Grosell et al., 2006b). A chronic value of 10 µg Pb/L obtained in 28-day exposures of 2-month-old fatmucket mussel, *L. siliquoidea* juveniles was the lowest genus mean chronic value ever reported for Pb (Wang et al., 2010f). The 96-hour EC_{50} values for 5-day-old juveniles in two separate toxicity tests with this species were 142 and 298 µg Pb/L (mean EC_{50} 220 µg Pb/L).

Recent studies (Sections 6.4.5.2 and 6.4.6) have further elucidated the role of water quality on Pb toxicity. In freshwater invertebrates some effects were observed at concentrations occasionally encountered in U.S. surface waters (Table 6-2). In a 7-day exposure of the cladoceran *C. dubia* to 50 to 500 µg Pb/L, increased DOC leads to an increase in mean EC_{50} for reproduction ranging from approximately 25 µg Pb/L to >500 µg Pb/L (Mager et al., 2011a). The 48-hour LC_{50} values for the cladoceran *C. dubia* tested in eight natural waters across the U.S. varied from 29 to 1,180 µg Pb/L and were correlated with DOC (Esbaugh et al., 2011).

Additional new evidence reviewed in Sections 6.4.5.2 and 6.4.6 for effects near the upper range of concentrations of Pb available from surveys of U.S. surface waters include studies with rotifer, midge and mayfly species. The freshwater rotifer *E. dilatata* 48 hour LC_{50} was 35 µg Pb/L using neonates hatched from asexual eggs (Arias-Almeida and Rico-Martínez, 2011). An EC_{20} for reduced growth and emergence of the midge *C. dilutus* was reported to be 28 µg Pb/L, observed in a 55-day exposure, while the same species had a 96-hour LC_{50} of 3,323 µg Pb/L (Mebane et al., 2008) The EC_{10} for molting in the mayfly *B. tricaudatus* was 37 µg Pb/L (Mebane et al., 2008). All of these effect concentrations provide additional evidence for Pb effects on freshwater invertebrates.

Vertebrates

For freshwater fish (Sections 6.4.5.3 and 6.4.6), most recent studies available since the 2006 Pb AQCD, were conducted with fathead minnow, *P. promelas*, or rainbow trout, *O. mykiss*. In a series of 96-hour acute toxicity tests with fathead minnow conducted in a variety of natural waters across North America, LC_{50} values ranged from 41 to 3,598 µg Pb/L (Esbaugh et al., 2011). Reproductive effects associated with water quality parameters were also noted with this species (Mager et al., 2010). In trout, no effects of

Pb were observed in dietary studies. In chronic aqueous exposures with trout the following endpoints were reported: NOEC=24 µg Pb/L, EC_{10}=26 µg Pb/L, EC_{20}=34 µg Pb/L, and LC_{50}=55 µg Pb/L. In a separate test with the same species an NOEC=8 µg Pb/L, EC_{10}=7µg Pb/L, EC_{20}=102 µg Pb/L and LC_{50}=120 µg Pb/L were reported. In acute tests with rainbow trout run concurrently with the chronic tests, 96-hour LC_{50} values were 120 and 150 µg Pb/L, respectively (Mebane et al., 2008). These reported effects provide additional evidence for toxicity of Pb to fish and chronic NOEC and EC_{10} values reported for trout, a sensitive species, are within the upper range of Pb currently reported in U.S. surface waters (Table 6-2).

In Section 6.4.5.3, a study with the frog *R. pipiens* exposed nominally to Pb, tissue concentrations were quantified at the end of the study and found to be in the range of Pb tissue concentrations in wild-caught tadpoles. Growth rate was significantly slower in the 100 µg Pb/L nominal concentration and more than 90% of tadpoles developed lateral spinal curvature. Time to metamorphosis was also delayed at this treatment level.

Food Web

In the 2006 Pb AQCD, trophic transfer of Pb through aquatic food chains was considered to be negligible. Concentrations of Pb in the tissues of aquatic organisms were generally higher in algae and benthic organisms than in higher trophic-level consumers indicating that Pb was bioaccumulated but not biomagnified (U.S. EPA, 2006c; Eisler, 2000). Some studies published since the 2006 Pb AQCD, (see Section 6.4.4.4) support the potential for Pb to be transferred in aquatic food webs, while other studies indicate that Pb concentration decreases with increasing trophic level (biodilution).

Community and Ecosystem Effects

New evidence of effects of Pb at the community and ecosystem levels of biological organization reviewed in Section 6.4.7 include shift in community composition in macrophytes. Effects on reproduction, growth or survival (summarized in Table 6-5) may lead to effects at the population-level of biological organization and higher. Additional evidence for community and ecosystem level effects of Pb have been observed primarily in microcosm studies or field studies with other metals present.

6.4.12 Causal Determinations for Pb in Freshwater Systems

In the following sections, organism-level effects on reproduction and development, growth and survival are considered first since these endpoints can lead to effects at the

population level or above and are important in ecological risk assessment. Neurobehavioral effects are considered next followed by sub-organismal responses (hematological effects, physiological stress) for which Pb has been shown to have an impact in multiple species and across taxa, including humans. Causal determinations for terrestrial, freshwater and saltwater ecological effects are summarized in Table 6-3.

6.4.12.1 Reproductive and Developmental Effects-Freshwater Biota

Evaluation of the findings in previous Pb AQCDs and recent literature on Pb effects in aquatic fauna indicates that exposure to Pb is associated with reproductive effects at or near ambient concentrations of this metal (Table 6-2) in some freshwater species. Impaired fecundity at the organismal level can result in a decline in abundance and/or extirpation of populations, decreased taxa richness, and decreased relative or absolute abundance at the community level (Suter et al., 2005; U.S. EPA, 2003a). Various endpoints have been measured in freshwater organisms to assess the effect of Pb on fecundity, development and hormone homeostasis. However, there are typically only limited studies available from different taxa. Recent evidence available since the 2006 Pb AQCD for effects of Pb on reproductive endpoints in freshwater invertebrates and vertebrates is summarized in Table 6-5.

There are no studies reviewed in the ISA or previous Pb AQCDs on development and reproductive effects of Pb in freshwater aquatic algae or macrophytes.

Experimental data from freshwater invertebrates provide evidence for increasing reproductive effects associated with increasing exposure to Pb. The exposure-response relationship is used in judging causality (Table I of the Preamble). Reproductive effects of Pb in freshwater aquatic invertebrates are well-characterized in previous Pb AQCDs, the draft *Ambient Aquatic Life Water Quality Criteria for Lead* report (U.S. EPA, 2008b), and in the current ISA and have been observed at or near current ambient concentrations (median 0.5 µg Pb/L, range 0.04 to 30 µg Pb/L) (U.S. EPA, 2006c) in some species in laboratory exposures. In the 1986 Pb AQCD reproductive effects were reported to begin at 19 µg Pb/L for the freshwater snail *Lymnaea palustris* and 27 µg Pb/L for Daphnia sp. (U.S. EPA, 1986b). In the 2006 Pb AQCD (U.S. EPA, 2006b) the number of neonates per surviving adult was significantly decreased in the amphipod *H. azteca* during chronic 42-day exposures to Pb (Besser et al., 2005). In the group exposed to Pb in water-only exposures, the LOEC for reproductive effects was 16 µg Pb/L while in amphipods receiving both water-borne and dietary Pb the LOEC for reproduction was 3.5 µg Pb/L.

New evidence in freshwater invertebrates (Table 6-5 and Section 6.4.5.2) show consistency of the observed association between reproductive endpoints and Pb exposure. In the freshwater rotifer *B. calyciflorus*, reproductive output was measured as total number of individuals and intrinsic growth rate. The EC_{20} for number of rotifers was 125 µg Pb/L and the 48 hour EC_{20} for intrinsic rate of population increase was 307 µg Pb/L with an NOEC of 194 µg Pb/L (Grosell et al., 2006b). In a 7-day exposure of the cladoceran *C. dubia* to 50 to 500 µg Pb/L, increased DOC leads to an increase in mean EC_{50} for reproduction ranging from approximately 25 µg Pb/L to >500 µg Pb/L (Mager et al., 2011a). Additional reproductive impairment endpoints for freshwater cladocerans are reported in Table 6 of the draft *Ambient Aquatic Life Water Quality Criteria for Lead* report (U.S. EPA, 2008b). It is not clear how these laboratory-derived values for freshwater invertebrates compare to Pb exposures in natural systems due to the role of modifying factors (i.e., pH, hardness, and DOC) which affect Pb speciation and bioavailability, however, results under controlled conditions have consistently shown reproductive effects of Pb in sensitive taxa (amphipods, cladocerans) at concentrations at or near Pb quantified in freshwater environments.

In freshwater aquatic vertebrates there is evidence for reproductive and developmental effects of Pb. Pb exposure in frogs has been demonstrated to delay metamorphosis, decrease larval size and produce skeletal malformations. For example, in northern leopard frog *R. pipiens* exposed to nominal concentrations of 100 µg Pb/L from embryonic stage to metamorphosis, maximum swimming speed was significantly slower than other treatment groups and 92% of tadpoles exposed to 100 µg Pb/L had lateral spinal curvature (compared with 6% in the control) (Chen et al., 2006b). Pb tissue concentrations were quantified in frogs following exposure and fell within the range of tissue concentrations in wild-caught tadpoles.

The weight of evidence for reproductive and developmental effects in freshwater vertebrates is from studies with fish. Pb AQCDs have reported developmental effects in fish, specifically spinal deformities in brook trout (*Salvelinus fontinalis*) exposed to 119 µg Pb/L for three generations (U.S. EPA, 1977), and in rainbow trout as low as 120 µg Pb/L (U.S. EPA, 1986b). Reproductive behaviors of fathead minnows including reduced time spent in nest preparation by males, increased interspawn periods and reduced oviposition by females was observed following a 4-week exposure to 500 µg Pb/L (Weber, 1993). In the 2006 Pb AQCD (U.S. EPA, 2006b), decreased spermatocyte development in rainbow trout was reported at 10 µg Pb/L and, in fathead minnow testicular damage occurred at 500 µg Pb/L. In a recent study, reproductive effects in fathead minnows were influenced by water chemistry parameters (alkalinity and DOC) in breeding exposures following 300 day chronic toxicity testing with Pb (Mager et al., 2010). Specifically, in fish treated in both 35 and 120 µg Pb/L with HCO_3^-

and with 120 µg Pb/L with DOC, total reproductive output was decreased and average egg mass production increased as compared to egg mass size in controls and in low HCO_3^- and DOC treatments with Pb. No significant differences were present between treatments in egg hatchability. In a feeding study, Reproductive performance was unaffected in zebrafish exposed to Pb-via consumption of contaminated prey (Boyle et al., 2010). In fish, there is evidence for alteration of steroid profiles and additional reproductive parameters although most of the available studies were conducted using nominal concentrations of Pb.

Observations of Pb toxicity to reproductive and developmental endpoints in freshwater fauna are further supported by evidence in terrestrial invertebrates and vertebrates (Section 6.3.12.1), marine invertebrates (Section 6.4.21.1) and from laboratory animals (Section 4.8). Pb appears to affect multiple endpoints associated with reproduction and development in aquatic invertebrates and vertebrates. A few sensitive invertebrate taxa (amphipods, cladocerans) have been identified where effects are observed in laboratory studies at concentrations of Pb that occur in the environment. Overall, there is a dearth of information on reproductive effects of Pb in natural environments; however, the weight of evidence is sufficient to conclude that there is a causal relationship between Pb exposures and developmental and reproductive effects in freshwater invertebrates and vertebrates. In freshwater plants, the evidence is inadequate to conclude that there is a causal relationship between Pb exposures and plant developmental and reproductive effects.

6.4.12.2 Growth Effects-Freshwater Biota

Alterations in the growth of an organism can impact population, community and ecosystem level variables. Growth is a commonly measured endpoint in aquatic plants, however, reported effects typically occur at concentrations that exceed Pb quantified in freshwater habitats. Growth effects of Pb on plants include visible growth responses and reduction of photosynthetic rate, inhibition of respiration, cell elongation, root development or premature senescence (U.S. EPA, 1986b). In the 2006 Pb AQCD (U.S. EPA, 2006b), both freshwater algae and plants had EC_{50} values for growth in the range of 1,000 to >100,000 µg Pb/L with minimal inhibition of growth observed as low as 100 µg Pb/L (U.S. EPA, 2006c). The most sensitive aquatic macrophyte reported in the 2006 Pb AQCD was *A. pinnata* with an EC_{50} for relative growth rate of 1,100 µg Pb/L following a 4-day exposure to Pb (Gaur et al., 1994). An LOEC of 25 µg Pb/L for reduced chlorophyll in Coontail (*Ceratophyllum demersum*), and 50 µg Pb/L in Cattail (*T. latifolia*) following 12-day exposure to Pb (as Pb acetate) were the lowest reported concentrations of growth-related effects in freshwater plants in the draft *Ambient Aquatic*

Life Water Quality Criteria for Lead report (U.S. EPA, 2008b), and were near the upper range of Pb values reported from sampling of U.S. surface waters (Table 6-2). Additional growth studies in freshwater algae and plants summarized in Table 6 of the draft *Ambient Aquatic Life Water Quality Criteria for Lead* report and Table 6-5 of the present document report growth effects in laboratory studies at concentrations that exceed measured levels of Pb in the aquatic environment (U.S. EPA, 2008b).

Most of the evidence for growth effects of Pb in freshwater biota is for invertebrates. Some of these studies report inhibition of growth in sensitive species occurring at or near the current upper range of Pb in surface waters (median 0.50 µg Pb/L, range 0.04 to 30 µg Pb/L) (U.S. EPA, 2006c). Growth effects of Pb on aquatic invertebrates were reviewed in the draft *Ambient Aquatic Life Water Quality Criteria for Lead* report (U.S. EPA, 2008b) and in the 2006 Pb AQCD. The lowest reported LOEC for growth in the 2006 Pb AQCD (16 µg Pb/L) was in amphipods (*H. azteca*) in a 42-day chronic exposure (Besser et al., 2005).

Recent studies provide additional evidence for effects on growth of freshwater aquatic invertebrates at ≤ 10 µg Pb/L. Growth effects observed in invertebrates underscores the importance of lifestage to overall Pb sensitivity. In general, juvenile organisms are more sensitive than adults. Growth of juvenile freshwater snails *L. stagnalis* was inhibited below the lowest concentration tested resulting in an EC_{20} <4 µg Pb/L (Grosell and Brix, 2009; Grosell et al., 2006b). In the same study, the NOEC was 12 µg Pb/L and the LOEC was 16 µg Pb/L. The authors indicated that the observed effect level for Pb was very close to the current U.S. EPA water quality criteria for Pb (3.3 µg Pb/L normalized to test water hardness) (Grosell and Brix, 2009). In the freshwater mussel, fatmucket (*L. siliquoidea*) juveniles were the most sensitive lifestage (Wang et al., 2010f). In this study, growth of juvenile mussels at the end of a 28-day exposure in 17 µg Pb/L was significantly reduced from growth in the controls. A chronic value of 10 µg Pb/L in 2-month-old fatmucket juveniles was the lowest genus mean-chronic value ever reported for Pb. The EC_{10} and EC_{20} for reduced growth and emergence of the midge *C. dilutus* in a 55-day exposure were 28 µg Pb/L and 55 µg Pb/L, respectively, while the same species had a 96-hour LC_{50} of 3,323 µg Pb/L (Mebane et al., 2008) The EC_{10} and EC_{20} for molting in the mayfly *B. tricaudatus* were 37 µg Pb/L and 66 µg Pb/L, respectively (Mebane et al., 2008). In natural freshwater systems the effects of Pb are influenced by additional factors (i.e., pH, hardness, and DOC) which may modulate the toxicity of Pb observed under laboratory conditions.

Evidence for growth effects of Pb in freshwater aquatic vertebrates is limited to a few studies in amphibians and fish. In the 2006 Pb AQCD growth effects of Pb were reported in frogs at concentrations typically higher than currently found in the environment. A

recent study supports findings of growth effects in frogs and suggests that these effects may be occurring at lower concentrations: the growth rate of tadpoles of the northern leopard frog exposed nominally to 100 µg Pb/L from embryo to metamorphosis was slower than the growth rate of the controls (Chen et al., 2006b). In this study, Pb concentrations in the tissues of tadpoles were quantified and the authors reported that they were within the range of reported tissue concentrations reported in wild-caught populations.

Reports of Pb-associated growth effects in freshwater fish are inconsistent (Mager, 2012). In a review cited in the 2006 Pb AQCD, general symptoms of Pb toxicity in fish included growth inhibition (Eisler, 2000) however, other studies with Pb have shown no effects on growth (Mager, 2012). In the studies reviewed for the current ISA no growth effects were observed in fish exposed to Pb via dietary intake. Recent aqueous exposure studies with fathead minnows showed significant increases in body length and body mass following chronic low Pb exposure, however, the authors noted that some effects were observed in tanks with high mortality early in the exposure (Mager and Grosell, 2011; Grosell et al., 2006a). Other studies with fathead minnows have shown growth reductions with Pb exposure, however, concentrations of observed effects typically exceeded the 96-hour LC_{50} value (Mager, 2012; Mager et al., 2010; Grosell et al., 2006a). Two 60-day early lifestage tests with rainbow trout showed differences in LOEC for reduced growth (Mebane et al., 2008). In the first test, a 69-day exposure, the LOECs for mortality and reduced growth were the same (54 µg Pb/L). In the second test, a 62-day exposure of Pb to rainbow trout, the LOEC for fish length was 18 µg Pb/L with an EC_{20} of >87 µg Pb/L.

Evidence of effects of Pb exposure on growth in terrestrial plants (Section 6.3.12.2) is highly coherent with evidence from freshwater plants. Although there is a lack of evidence in freshwater plants for growth effects at concentrations of Pb typically encountered in U.S. surface waters, several studies suggest that minimal growth inhibition can occur within one to two orders of magnitude of the reported range for freshwater. Due to the concentration-response relationship observed between Pb exposure and freshwater plants, growth is likely impacted at lower, more ecologically relevant EC_{10} or LOEC values, than the typically reported EC_{50} values which may not be suitable for a maintaining a sustainable population.

There is a large body of evidence to support growth effects of Pb on aquatic plants at concentrations that greatly exceed those typically found in U.S. surface waters. Less evidence is available at current concentrations of Pb measured in U.S. surface waters and within one to two orders of magnitude above the range of these measured values. The available evidence is, however, sufficient to conclude that a causal relationship is likely to exist between Pb exposures and growth effects in freshwater plants. The evidence is

sufficient to conclude that there is a causal relationship between Pb exposures and growth effects in aquatic invertebrates. Available evidence is inadequate to conclude that there is a causal relationship between Pb exposures and growth effects in aquatic vertebrates.

6.4.12.3 Survival-Freshwater Biota

The relationship between Pb exposure and survival has been well demonstrated in freshwater species as presented in Section 6.4.5 and Table 6-5 of the present document and in the previous Pb AQCDs. Pb exposure can either result in direct lethality or produce sublethal effects that diminish survival probabilities. Survival is a biologically important response that can have a direct impact on population size. The concentration at which there is 50% mortality of test organisms (LC_{50}) is one of the most commonly reported measures of toxicity. LC_{50} is more sensitive to changes in exposure than estimates of lethal concentration for other percentages of the population such as LC_{10} (the concentration at which there is 10% mortality), and can thus be estimated with greater precision. However, LC_{50} alone may be less informative for consideration of effects at ecologically-relevant concentrations. It is most commonly used to describe acute toxicity, whereas Pb effects on ecosystem receptors may also be characterized as a result of chronic and cumulative exposures. Furthermore, a scenario in which 50% of a population does not survive is likely not a sustainable population. From the LC_{50} data on Pb in this review and previous Pb AQCDs, a wide range of sensitivity to Pb is evident across taxa and within genera. However, the LC_{50} is usually much higher than current environmental levels of Pb in the U.S, even though physiological dysfunction that adversely impacts the fitness of an organism often occurs at concentrations well below lethal ones. When available, LC_{10}, LC_{20}, NOEC, or LOEC are therefore reported.

There are no studies reported in the previous Pb AQCDs or the current ISA for aquatic plants that indicate phytotoxicity at current concentrations of Pb in freshwater environments.

There are considerable data available on toxicity of Pb to aquatic invertebrates as reviewed in the previous Pb AQCDs and the draft *Ambient Aquatic Life Water Quality Criteria for Lead* report (U.S. EPA, 2008b, 1985). Table AX7-2.4.1 from the 2006 Pb AQCD summarizes LC_{50} data and other endpoints for freshwater and marine invertebrates (U.S. EPA, 2006c). Recent studies available since the 2006 Pb AQCD and *the draft Ambient Aquatic Life Water Quality Criteria for Lead* document, that report mortality data are summarized in Table 6-5. Freshwater invertebrates are generally more sensitive to Pb exposure than other taxa, with survival impacted in a few species at or near concentrations that are encountered in aquatic environments (Table 6-2). These

impacted species may include candidate or endangered species. For example, the freshwater mussel *L. rafinesqueana* (Neosho mucket), is a candidate for the endangered species list. The EC_{50} for foot movement (a measure of viability) for newly transformed juveniles of this species was 188 µg Pb/L. (Wang et al., 2010f).

Most of the evidence for Pb effects on survival in freshwater invertebrates is from sensitive species of gastropods, amphipods, cladocerans and rotifers (Sections 6.4.5.2 and 6.4.6). In some of these organisms, increased mortality is observed in the upper range of Pb concentration values found in surveys of U.S. surface waters (median 0.50 µg Pb/L, range 0.04 to 30 µg Pb/L) (U.S. EPA, 2006c), although the toxicity of Pb is highly dependent upon water quality variables such as DOC, hardness and pH. In the 1986 Pb AQCD, increased mortality was reported in the freshwater gastropod *Lymnaea palutris* at Pb concentration as low as 19 µg Pb/L effectively reducing total biomass production (Borgmann et al., 1978). Toxicity testing with amphipods under various water conditions indicate these organisms are sensitive to Pb at <10 µg Pb/L (U.S. EPA, 2006c) and the present document). A 7 day LC_{50} of 1 µg Pb/L was observed in soft water with the amphipod *H. azteca (*Borgmann et al., 2005*)*. In this same species, the 96-hour LC_{50} for Pb at pH of 5 was 10 µg Pb/L (Mackie, 1989). In 42-day chronic exposures of *H. azteca* exposed to Pb via water and diet, the LC_{50} was 16 µg Pb/L (Besser et al., 2005). At higher pH and water hardness, amphipods are less sensitive to Pb (U.S. EPA, 2006c). In a series of 48 hour acute toxicity tests with the cladoceran *C. dubia* conducted in a variety of natural waters across North America, LC_{50} values ranged from 29 to 1,180 µg Pb/L (NOEC range 18 to <985 µg Pb/L) and were most significantly influenced by DOC and water ionic strength (Esbaugh et al., 2011). In the 2006 Pb AQCD the range of 48 hour LC_{50} values for *C. dubia* were reported from 280 to >2,700 µg Pb/L when tested at varying pH levels (U.S. EPA, 2006c). In the rotifer genus *Lecane*, a 22-fold difference in LC_{50} values was observed in 48-hour exposure to Pb between *L. hamata, L. luna* and *L. quadridentata*. (Pérez-Legaspi and Rico-Martínez, 2001). *L. luna* was most sensitive to Pb toxicity with a 48-hour LC_{50} of 140 µg Pb/L. In neonate rotifers, *E. dilatata* the 48-hour LC_{50} was 35 µg Pb/L (Arias-Almeida and Rico-Martínez, 2011). A wide range of LC_{50} values were reported for chironomid species (Table 6-5), however, the available evidence suggests these freshwater invertebrates are less sensitive to Pb than amphipods, cladocerans and rotifers. Other freshwater invertebrates such as odonates may be tolerant of Pb concentrations that greatly exceed concentrations of Pb reported in environmental media. Some invertebrates are able to detoxify Pb such as through sequestration of Pb in the exoskeleton which is subsequently molted.

There is considerable historic information on Pb toxicity to freshwater fish. Early observations from mining areas where Pb and other metals were present indicated local extinction of fish from streams (U.S. EPA, 1977). The lowest LC_{50} for fish reported in

the 1977 Pb AQCD was 1,000 µg Pb/L in soft water for rainbow trout *O. mykiss* (reclassified from *Salmo gairdneri*) following 96-hour exposure to Pb (U.S. EPA, 1977). Additional LC_{50} values for freshwater fish are summarized in the 1985 *Ambient Water Criteria for Lead* (U.S. EPA, 1985) and the draft *Ambient Aquatic Life Water Quality Criteria for Lead* report (U.S. EPA, 2008b). An LC_{50} of 236 µg Pb/L adjusted to a total hardness of 50 mg/L $CaCO_3$ was reported for *O. mykiss* in the draft *Ambient Aquatic Life Water Quality Criteria for Lead* report.

More recently reviewed studies using fish have considered the role of water quality variables and bioavailability on Pb toxicity (Table 6-5). Higher toxicity tends to occur in acidic waters where more free-Pb ion is available for uptake. The interactive effects of Pb concentration and water quality variables on toxicity may result in equivalent toxicity for a broad range of Pb concentrations. In a series of 96-hour acute toxicity tests with juvenile fathead minnow conducted in a variety of natural waters across North America, LC_{50} values ranged from 41 to 3,598 µg Pb/L and no Pb toxicity occurred in three highly alkaline waters (Esbaugh et al., 2011). In the 2006 Pb AQCD, the 96-hr LC_{50} values in fathead minnow ranged from 810->5,400 µg Pb/L in varying pH and hardness (U.S. EPA, 2006c).

Decreased survival is also a function of age of the fish. Thirty day LC_{50} values for larval fathead minnows ranged from 4.5 to 1,903 µg Pb/L in varying concentrations of DOC, $CaSO_4$ and pH (Grosell et al., 2006a). In a recent study of rainbow trout fry at 2 to 4 weeks post swim-up, the 96-hour LC_{50} was 120 µg Pb/L at a hardness of 29 mg/L as $CaCO_3$, a value much lower than in testing with older fish (Mebane et al., 2008). In the same study, two chronic (>60 day) tests were conducted with rainbow trout and the NOECs for survival were 24 and 87 µg Pb/L and the LOECs were 54 and 125 µg Pb/L, respectively. In contrast to aqueous exposures, 30 day dietary studies with rainbow trout fathead minnow, and channel catfish fed a live diet of *L. variegatus* contaminated with Pb showed no statistically significant effects on survival (Erickson et al., 2010).

Freshwater fish are less sensitive to Pb than freshwater invertebrates, however, recent studies have highlighted the importance of considering pH, hardness, and additional modifying factors in assessing toxicity since effects can vary over several orders of magnitude. Fish mortalities occur above the concentrations of Pb encountered in U.S. surface waters although, in some cases, the observed effects may be just above the upper measured range of Pb in some aquatic environments (Table 6-2). Furthermore, although LC_{50} values are the most commonly reported, effects are occurring at lower concentrations. A more relevant indication of exposure impacts would be an LC_{10} or LOEC, however, these values are not always provided. The evidence is sufficient to conclude that there is a causal relationship between Pb exposures and survival in

freshwater invertebrates and vertebrates. The evidence is inadequate to conclude that there is a causal relationship between Pb exposures and survival in freshwater plants.

6.4.12.4 Neurobehavioral Effects-Freshwater Biota

Evidence from laboratory studies and limited data from field studies reviewed in this chapter, in the draft *Ambient Aquatic Life Water Quality Criteria for Lead* report, which updates the 1985 *Ambient Water Quality Criteria for Lead* (U.S. EPA, 1985), and in previous Pb AQCDs have shown effects of Pb on neurological endpoints in aquatic animal taxa. These include changes in behaviors that may decrease the overall fitness of the organism such as avoidance responses, decreased ability of an organism to capture prey or escape predators, and alterations in feeding behaviors. Evidence of alteration in behaviors at the level of the organism is a potential endpoint for effects at population or community levels of biological organization (U.S. EPA, 2003a).

In the 1977 Pb AQCD behavioral impairment of a conditioned response (avoidance of a mild electric shock) in goldfish was observed as low as 70 µg Pb/L (Weir and Hine, 1970). In the 2006 Pb AQCD several studies were reviewed in which Pb was shown to affect predator-prey interactions, including alteration in prey size choice and delayed prey selection in juvenile fathead minnows following 2-week pre-exposure to 500 µg Pb/L (Weber, 1996). In limited studies available on worms, snails, tadpoles, hatchling turtles and fish there is evidence that Pb may affect the ability to escape or avoid predation. For example, in the tubificid worm *T. tubifex* the 96 hour EC_{50} for immobilization was 42 µg Pb/L (Khangarot, 1991). Some organisms exhibit behavioral avoidance while others do not seem to detect the presence of Pb (U.S. EPA, 2006c). Additional behavioral endpoints reported in the draft *Ambient Aquatic Life Water Quality Criteria for Lead* document include an EC_{50} of 140 µg Pb/L for feeding inhibition in the freshwater cladoceran *C. dubia* and deceased learning acquisition in bullfrogs at 500 µg Pb/L (31.51 µg Pb/L adjusted to a total hardness of 50 mg/L $CaCO_3$). All of these effects occur at concentrations that exceed Pb concentration values found in surveys of U.S. surface waters although within the range of Pb detected near some mining-disturbed areas (Table 2-11).

Recent information since the 2006 Pb AQCD provides evidence for Pb impacts on behaviors that may affect feeding and predator avoidance in freshwater environments at concentrations near the range of Pb detected in U.S. surface waters (Table 6-2 and Section 6.4.5.3). Prey capture ability was decreased in 10 day old fathead minnow larvae born from adult fish exposed to 120 µg Pb/L for 300 days, then subsequently tested in a 21-day breeding assay (Mager et al., 2010). Another study in fish reported effects at

low µg Pb/L concentration, however, the findings are not considered as strong evidence for causality since exposure concentrations in water were not analytically verified. Specifically, zebrafish embryos exposed nominally to concentrations of Pb (2.0 to 6.0 µg Pb/L) until 24 hours post-fertilization and then subsequently tested as larvae exhibited decreased startle response time and altered pattern of escape swimming (Rice et al., 2011). In adult fish raised from the exposed embryos (6.0 µg Pb/L), the ability to detect visual contrast was degraded. Although this study was conducted with nominal concentration of Pb in media, uptake of Pb by embryos was quantified and more Pb was measured in tissues of embryos exposed to the higher concentration of Pb when compared to the lower exposure concentration. Additional studies are needed in fish to support these initial findings of effects on ecologically relevant behavioral impairments.

Findings in laboratory animals support the limited evidence for neurobehavioral effects of Pb in freshwater invertebrates and vertebrates. In animal toxicological studies Pb induced changes in learning and memory (Section 4.3.2.3). New behaviors induced by exposure to Pb reviewed in Chapter 4 that are relevant to effects of Pb observed in freshwater systems include effects on visual and auditory sensory systems and changes in structure and function of neurons and supporting cells in the brain. Mechanisms that include the displacement of physiological cations, oxidative stress and changes in neurotransmitters and receptors are also reviewed. Central nervous system effects in fish recognized in previous Pb AQCDs include effects on spinal neurons and brain receptors. New evidence from this review identifies the MAPKs ERK1/2 and $p38^{MAPK}$ as possible molecular targets for Pb neurotoxicity in catfish (Leal et al., 2006). Evidence in terrestrial ecosystems (Section 6.3.12.4) is not as extensive, but it is highly coherent with findings in aquatic ecosystems. Overall, the evidence from available studies on neurobehavioral effects of Pb in aquatic systems is limited, but sufficient to conclude that a causal relationship is likely to exist between Pb exposures and neurobehavioral effects in aquatic invertebrates and vertebrates.

6.4.12.5 Hematological Effects-Freshwater Biota

Hematological responses are commonly reported effects of Pb exposure in aquatic invertebrates and vertebrates. Anemia was recognized as a symptom of chronic Pb poisoning in fish in the 1977 Pb AQCD and has been subsequently reported in various fish species using common hematological endpoints (e.g., red blood cell counts, hematocrit, Hb concentrations) (Mager, 2012). In the 1986 Pb AQCD, hematological effects of Pb exposure on fish included decrease in red blood cells and inhibition of ALAD (U.S. EPA, 1986b). Inhibition of ALAD activity under various test conditions is reported in Table 6 of the the draft *Ambient Aquatic Life Water Quality Criteria for Lead*

report, for freshwater fish species (*O. mykiss* (Rainbow Trout), *S. fontinalis* (Brook Trout), *C. auratus* (Goldfish) and *Lepomis gibbosus* (Pumpkinseed)) (U.S. EPA, 2008b). In these studies, Rainbow Trout was the most sensitive with inhibition of ALAD activity reported in multiple studies within the upper range of Pb in surface waters of the U.S. (median 0.5 µg Pb/L, range 0.04 to 30 µg Pb/L) (U.S. EPA, 2006c).

Laboratory studies with freshwater invertebrates have also indicated considerable species differences in ALAD activity in response to Pb. For example, the concentration at which 50% ALAD inhibition was measured in the freshwater gastropod *B. glabrata* (23 to 29 µg Pb/L) was much lower than in the freshwater oligochaete *L. variegatus* (703 µg Pb/L) based on nominal exposure data (Aisemberg et al., 2005).

Findings in laboratory studies are additionally supported by evidence from field-collected organisms providing coherence to the observations of Pb effects on ALAD activity. In environmental assessments of metal-impacted habitats, ALAD is a recognized biomarker of Pb exposure (U.S. EPA, 2006c). ALAD activity is negatively correlated with total Pb concentration in freshwater bivalves, and lower ALAD activity has been correlated with elevated blood Pb levels in field-collected fish as well. Further evidence from the 2006 Pb AQCD and this review of Pb effects on ALAD enzymatic activity, including effects in bacteria, amphibians and additional field and laboratory studies on freshwater fish, confirms that the decreased activity in this enzyme is an indicator for Pb exposure across a wide range of taxa and that a common mode of action is likely for invertebrates and vertebrates. The finding that the hematological system is a target for Pb in natural systems is also supported by some evidence of Pb-induced alterations of serum profiles and changes in white blood cell counts in fish (U.S. EPA, 2006c) and amphibians. This evidence is strongly coherent with evidence from terrestrial vertebrates (Section 6.3.12.5). It is also coherent with observations from human epidemiologic and animal toxicology studies (Section 4.7) where there is consistent evidence that exposure to Pb induces adverse effects on hematological endpoints, including altered heme synthesis mediated through decreased ALAD and ferrochelatase activities, decreased red blood cell survival and function, and increased red blood cell oxidative stress. The overall weight of epidemiologic and toxicological evidence for humans was sufficient to conclude that a causal relationship exists between exposure to Pb and hematological effects (Section 4.7).

Based on observations in freshwater organisms and additionally supported by findings in terrestrial systems, saltwater invertebrates (Section 6.4.21.5), and by toxicological and epidemiologic evidence on human health effects, evidence is sufficient to conclude that there is a causal relationship between Pb exposures and hematological effects in freshwater vertebrates. Evidence is sufficient to conclude that a causal relationship is

likely to exist between Pb exposures and hematological effects in freshwater invertebrates.

6.4.12.6 Physiological Stress-Freshwater Biota

Building on the body of evidence presented in the 2006 Pb AQCD (U.S. EPA, 2006c) recent studies provide consistent and coherent evidence of upregulation of antioxidant enzymes and increased lipid peroxidation associated with Pb exposure within one or two orders of magnitude above current or ambient conditions in many species of freshwater plants, invertebrates and vertebrates. A few studies provide evidence of effects at concentrations of Pb encountered in some sediments of the U.S. (Table 6-2). In aquatic plants, increases of antioxidant enzymes with Pb exposure occur in algae, mosses, and floating and rooted aquatic macrophytes. Most available evidence for antioxidant responses in aquatic plants are from laboratory studies lasting from 2 to 7 days and at concentrations higher than typically found in the environment. However, data from transplantation experiments from non-polluted to polluted sites indicate that elevated enzyme activities are associated with Pb levels measured in sediments. For example, the freshwater macrophyte *Myriophyllum quitense* exhibited elevated antioxidant enzyme activity (glutathione-S-transferase, glutathione reductase, peroxidase) following transplantation in anthropogenically polluted areas containing elevated Pb concentrations. These were correlated with sediment Pb concentrations in the range of 5 to 23 mg Pb/g dry weight (Nimptsch et al., 2005).There is evidence for antioxidant activity in response to Pb exposure in freshwater invertebrates (i.e., bivalves). Markers of oxidative damage are also observed in fish, amphibians and mammals in laboratory studies. Across all organisms, there are differences in the induction of antioxidant enzymes that appear to be species-dependent.

Additional stress responses to Pb in a few aquatic invertebrates have been reported since the 2006 Pb AQCD, and included elevated heat shock proteins, osmotic stress, lowered metabolism and decreased glycogen levels associated with Pb exposure. Although these stress responses are correlated with Pb exposure, they are non-specific and may be altered with exposure to any number of environmental stressors. Heat shock protein induction has been observed in zebra mussels exposed to 500 µg Pb/L for 10 weeks (Singer et al., 2005). Crayfish exposed for 14 days to 500 µg Pb/L exhibited a Pb-induced hypometabolism under conditions of environmental hypoxia in the presence of the metal (Morris et al., 2005). Glycogen levels in the freshwater snail *B. glabrata* were significantly decreased following 96-hour exposures at 50 µg/L and higher (Ansaldo et al., 2006).

Evidence for stress responses observed in freshwater plants, invertebrates and vertebrates is coherent with findings in terrestrial species (Section 6.3.12.6) and saltwater invertebrates (Section 6.4.21.6). It is also coherent with evidence from human and experimental animal studies of oxidative stress following impairment of normal metal ion functions (Section 4.2.4). Upregulation of antioxidant enzymes and increased lipid peroxidation are considered to be reliable biomarkers of stress, and provide evidence that Pb exposure induces a stress response in those organisms which may increase susceptibility to other stressors and reduce individual fitness. Evidence is sufficient to conclude that a causal relationship is likely to exist between Pb exposures and physiological stress in freshwater aquatic plants, invertebrates and vertebrates.

6.4.12.7 Community and Ecosystem Level Effects-Freshwater Biota

Most direct evidence of community- and ecosystem-level effects in freshwater systems is from heavily contaminated sites where Pb concentrations are higher than typically observed environmental concentrations for this metal. Impacts of Pb on aquatic habitats that receive runoff from contaminated areas have been studied for several decades. For aquatic systems, the literature focuses on evaluating ecological stress from Pb originating from urban and mining effluents rather than atmospheric deposition. Ecosystem-level field studies are complicated by the confounding of Pb exposure with other factors such as the presence of trace metals and acidic deposition and by the variability inherent in natural systems. In natural systems, Pb is often found co-existing with other stressors, and observed effects may be due to cumulative toxicity.

In laboratory studies and simulated ecosystems, where it is possible to isolate the effect of Pb, this metal has been shown to alter competitive behavior of species, predator-prey interactions and contaminant avoidance. These dynamics may change species abundance and community structure at higher levels of ecological organization. Uptake of Pb into aquatic and terrestrial organisms and subsequent effects on mortality, growth, developmental and reproductive endpoints at the organism level are expected to have ecosystem-level consequences, and thus provide consistency and plausibility for causality in ecosystem-level effects.

In aquatic ecosystems, field studies reviewed in the 2006 Pb AQCD (summarized in Table AX7-2.5.2) and more recent studies report reductions of species abundance, richness or diversity. This is particularly the case for benthic macroinvertebrate communities where sources of Pb were mining or urban effluents, and Pb coexisted with other metals. The results often indicate a correlation between the presence of one or more

metals and the negative effects observed. For example, in the 2006 Pb AQCD, the Coeur d'Alene River watershed in Idaho, U.S. was used as a case study for Pb effects at the population and community level. Significant negative correlations were observed between Pb in water column and total taxa richness and EPT taxa richness in the river. In a simulated aquatic microcosm a reduction in abundance and richness of protozoan species was observed with increasing Pb concentration from 50 to 1,000 µg Pb/L (Fernandez-Leborans and Antonio-Garcia, 1988).

Since the 2006 Pb AQCD, there is further evidence for effects of Pb in sediment-associated communities. Sediment-bound Pb contamination appears to differentially affect members of the benthic invertebrate community, potentially altering ecosystem dynamics in small urban streams (Kominkova and Nabelkova, 2005). Although surface water Pb concentrations in monitored streams were determined to be very low, concentrations of the metal in sediment were high enough to pose a risk to the benthic community (e.g., 34 to 101 mg Pb/kg). These risks were observed to vary with benthic invertebrate functional feeding group, with collector-gatherer species exhibiting larger body burdens of heavy metals than benthic predators and collector-filterers.

In a recent study conducted since the 2006 Pb AQCD, changes to aquatic plant community composition have been observed in the presence of elevated surface water Pb concentrations at three lake sites impacted by mining effluents. The site with highest Pb concentration (103-118 µg Pb/L) had lowest number of aquatic plant species when compared to sites with lower Pb concentrations (78-92 µg Pb/L) (Mishra et al., 2008). Certain types of plants such as rooted and submerged aquatic plants may be more susceptible to aerially deposited Pb resulting in shifts in Pb community composition. High Pb sediment concentrations are linked to shifts in amphipod communities inhabiting plant structures.

Avoidance response to Pb exposure varies widely in different species and this could affect community composition and structure and species abundance. For example, frogs and toads lack avoidance response while snails and fish avoid higher concentrations of Pb (U.S. EPA, 2006c).

In the Annex to the 2006 Pb AQCD, the Coeur d'Alene River basin in Idaho was presented as a case study for a watershed impacted by Pb and other metals. A significant negative correlation was observed between Pb in water column (0.5 to 30 µg Pb/L) and total taxa richness, EPT taxa richness, and the number of metal-sensitive mayfly species (Maret et al., 2003). Additional lines of evidence including mine density, metal concentrations, and bioaccumulation in caddisfly tissue were included. Since the 2006 Pb AQCD, additional research at this site and model development has resulted in further characterization of the effects of Pb on waterfowl and other aquatic organisms in

this heavily contaminated ecosystem. Mean Pb concentrations in Coeur d'Alene sediment range from 14 to 5,009 mg Pb/kg dry weight (Spears et al., 2007). Modeling of sediment and Pb levels in waterfowl predict a sediment Pb effects range of 147-944 mg Pb/kg dry weight and a mortality effects level of 1,652 mg/kg dry weight (Spears et al., 2007). In a 6-week feeding study with mallard (*Anas platyrhynchos*) ducklings, ingestion of Pb-contaminated sediments from the Coeur d' Alene basin was shown to result in decreased brain growth and altered brain chemistry (Douglas-Stroebel et al., 2004). These findings support previous observations of altered behavior and hematological, hepatotoxic, and histopathological endpoints in waterfowl from Lake Coeur d'Alene that ingest Pb contaminated sediments and vegetation during feeding.

In addition to the evidence from microcosm and field studies presented above, effects on reproduction (Section 6.4.12.1), growth (Section 6.4.12.2) and survival (Section 6.4.12.3) have been clearly demonstrated in freshwater species. These endpoints can have effects at the population-level and community-level of biological organization which may lead to ecosystem-level impacts. Although the evidence is strong for effects of Pb on growth, reproduction and survival in certain species in experimental settings at or near the range of Pb concentrations reported in surveys of U.S. freshwater systems, considerable uncertainties exist in generalizing effects observed under small-scale, particular conditions up to predicted effects at the ecosystem level of biological organization. In many cases it is difficult to characterize the nature and magnitude of effects and to quantify relationships between ambient concentrations of Pb and ecosystem response due to presence of multiple stressors, variability in field conditions and to differences in Pb bioavailability at that level of organization. Bioavailability of Pb is influenced by pH, alkalinity, total suspended solids, and DOC among other factors and can vary greatly in natural environments. Nevertheless, evidence of ecosystem effects in aquatic systems is coherent with similar evidence in terrestrial systems, and based on the cumulative evidence from laboratory studies and field observations, a causal relationship is likely to exist between Pb exposures and the alteration of species richness, species composition and biodiversity in freshwater aquatic ecosystems.

6.4.13 Introduction to Bioavailability and Biological Effects of Pb in Saltwater Ecosystems

Saltwater ecosystems include salt marsh, estuaries, embayments, beaches, and other coastal areas; and encompass a range of salinities from just above that of freshwater to that of seawater. These ecosystems may receive Pb contributions from direct atmospheric deposition and/or via runoff from terrestrial systems. A range of 0.01 to 27 µg Pb/L including coastal areas, estuaries and open ocean was reported by Sadiq (1992) with the

higher values associated with sites involving human activity, however, these values are not specific to the U.S. (Table 6-2). In an earlier publication, levels of Pb in the North Atlantic and North Pacific surface waters ranged from 0.005 to 0.4 µg Pb/L but the range of values in coastal waters and estuaries were approximately equal to the range of Pb in freshwater (Leland and Kuwabara, 1985). Additional information on Pb levels in water is available in Sections 6.2.3 and 2.6. The 2006 Pb AQCD provided an overview of regulatory considerations for water and sediments in addition to consideration of biological effects and major environmental factors that modify the response of marine organisms to Pb exposure. Regulatory guidelines for Pb in saltwater have not changed since the 2006 Pb AQCD and are summarized below. This section is followed by new information on bioavailability and biological effects of Pb in saltwater since the 2006 Pb AQCD.

The most recent ambient water quality criteria for Pb in saltwater were released in 1985 (U.S. EPA, 1985) by the EPA Office of Water which employed empirical regressions between observed toxicity and water hardness to develop hardness-dependent equations for acute and chronic criteria. These criteria are published pursuant to Section 304(a) of the Clean Water Act and provide guidance to states and tribes to use in adopting water quality standards for the protection of aquatic life and human health in surface water. The ambient water quality criteria for Pb are currently expressed as a criteria maximum concentration (CMC) for acute toxicity and criterion continuous concentration (CCC) for chronic toxicity (U.S. EPA, 2009b). In saltwater, the CMC is 210 µg Pb/L and the CCC is 8.1 µg Pb/L. The 2006 Pb AQCD summarized two approaches for establishing sediment criteria for Pb based on either bulk sediment or equilibrium partitioning as reviewed in the present document in Section 6.4.2.

In the following sections, recent information available since the 2006 Pb AQCD on Pb in marine and estuarine ecosystems will be presented. Throughout the sections, brief summaries of conclusions from the 1977 Pb AQCD (U.S. EPA, 1977), the 1986 Pb AQCD (U.S. EPA, 1986b) and the 2006 Pb AQCD (U.S. EPA, 2006b) are included where appropriate. Recent research on the bioavailability and uptake of Pb into saltwater organisms including plants, invertebrates and vertebrates is presented in Section 6.4.14. Toxicity of Pb to marine flora and fauna including growth, reproductive and developmental effects (Section 6.4.15) are followed with data on exposure and response of saltwater organisms (Section 6.4.16). Responses at the community and ecosystem levels of biological organization are reviewed in Section 6.4.17 followed by characterization of sensitivity and vulnerability of saltwater ecosystem components (Section 6.4.18) and a discussion of ecosystem services (Section 6.4.19). The saltwater sections conclude with a synthesis of the new data for Pb effects on saltwater plants,

invertebrates and vertebrates (Section 6.4.20) and causal determinations based on evidence from previous Pb AQCDs and recent studies (Section 6.4.21).

6.4.14 Bioavailability of Pb in Saltwater Systems

Bioavailability was defined in the 2006 Pb AQCD as "the proportion of a toxin that passes a physiological membrane (the plasma membrane in plants or the gut wall in animals) and reaches a target receptor (cytosol or blood)". In 2007, EPA took cases of bioactive adsorption into consideration and revised the definition of bioavailability as "the extent to which bioaccessible metals absorb onto, or into, and across biological membranes of organisms, expressed as a fraction of the total amount of metal the organism is proximately exposed to (at the sorption surface) during a given time and under defined conditions" (U.S. EPA, 2007c)

Factors affecting bioavailability of Pb to marine organisms are the same as those in freshwater systems (Sections 6.4.2 and 6.4.4). However, although routes of exposure and physiological mechanisms for storage and excretion influence uptake of metals by all organisms, they may be different in marine organisms, particularly for ion transport mechanisms (Niyogi and Wood, 2004). Marine environments are characterized by higher levels of ions, such as Na^+, Ca^{2+}, and Mg^{2+}, which compete for potential binding sites on biotic ligands such as gills, thereby generally reducing the effective toxicity of metal ions as compared to freshwater environments. However, because the concentrations of these ions are relatively constant, bioavailability may be more predictable in marine systems that are little influenced by freshwater than in freshwater systems, varying mostly with amount and type of dissolved organic matter. In estuaries and embayments, changing salinities and proximity to anthropogenic loading of pollutants add to the complexity of predicting Pb speciation in these dynamic systems. BLMs (Figure 6-3) now being developed for marine organisms are functionally similar to those applied to freshwater organisms (Section 6.4.4).

Although in freshwater systems the presence of humic acid is considered to reduce the bioavailable fraction of metals in freshwater, there is evidence that DOC/DOM does not have the same effect on free Pb ion concentration in marine systems (see Section 6.4.2.4 for detailed discussion). For the sea urchin *P. lividus*, the presence of humic acid increased both the uptake and toxicity of Pb possibly by enhancing uptake of Pb via membrane Ca^{2+} channels (Sanchez-Marin et al., 2010b). This also was observed in the marine diatom *Thalassiosira weissflogii*, where humic acids absorbed to cell surfaces increased metal uptake (Sanchez-Marin et al., 2010b). Formation of a ternary complex that is better absorbed by biological membranes was another proposed mechanism that

could describe the increased bioavailability to marine invertebrates of Pb bound to humic acid (Sánchez-Marín et al., 2007).

Sanchez-Marin et al. (2011) subsequently have shown that different components of DOM have different effects on Pb bioavailability in marine systems. Their initial research using Aldrich humic acid found that increasing humic acid concentrations increased Pb uptake by mussel gills and increased toxicity to sea urchin larvae in marine environments (Sánchez-Marín et al., 2007). In contrast, a subsequent investigation found that fulvic acid reduced Pb bioavailability in marine water (Sánchez-Marín et al., 2011). The contradictory effects of different components of DOM on marine bioavailability likely reflect their distinct physico-chemical characteristics. More hydrophobic than fulvic acid, humic acid may adsorb directly with cell membranes and enhance Pb uptake through some (still unidentified) mechanism (Sánchez-Marín et al., 2011). Pb AVS-measurements were also determined to accurately predict uptake by mussels (*Mytilus* sp.) in the presence of 2.5 to 20 mg/L fulvic acid (Sánchez-Marín et al., 2011). However, the effects of DOM on Pb bioavailability to mussels were underpredicted by AVS Pb concentration measurements, potentially as a result of adsorption of DOM-Pb complexes.

Based on the above, BLMs (see Section 6.4.4 and Figure 6-3) used to predict bioavailability of Pb to aquatic organisms (Di Toro et al., 2005), may require modifications for application to marine organisms. Of particular importance is the finding that in marine aquatic systems, surface water DOM was found to increase (rather than decrease) uptake of Pb by fish gill structures, potentially through the alteration of membrane Ca^{2+} channel permeability. Veltman et al. (2010) proposed integrating BLM and bioaccumulation models in order to more accurately predict metal uptake by fish and invertebrates, and calculated metal absorption efficiencies for marine fish species from both types of models. They noted that affinity constants for Ca^{2+}, Cd, Cu, Na, and Zn were highly similar across different aquatic species, including fish and invertebrates (Veltman et al., 2010). These findings suggest that the BLM can be integrated with bioaccumulation kinetics to account for both environmental chemical speciation and biological and physiological factors in both marine and freshwater systems.

6.4.14.1 Saltwater Plants and Algae

In the 1977 Pb AQCD, the cordgrass *Spartina alterniflora* was found to reduce by a small amount the quantity of Pb in sediments (U.S. EPA, 1977). Limited data on marine algal species reviewed in the 1986 Pb AQCD and 2006 Pb AQCD provided additional evidence for Pb uptake. Recent data available since the 2006 Pb AQCD includes Pb bioaccumulation studies conducted with five species of marine algae, (*Tetraselmis chuii*,

Rhodomonas salina, *Chaetoceros* sp., *Isochrysis galbana*, and *Nannochloropsis gaditana*). In this study it was demonstrated that bioaccumulation rates varied with species following 72-hour exposure to Pb. *I. galbana* accumulated the lowest concentrations of Pb (0.01 and 0.6 pg Pb/cell at water concentrations of 51 and 6,348 µg Pb/L), while *Chaetoceros* sp. was observed to be the most efficient Pb bioaccumulator, adsorbing 0.04 and 54 pg Pb/cell at 51 and 6,348 µg Pb/L (Debelius et al., 2009).

Recent uptake studies of Pb in plants associated with marine environments are also available. The roots of two salt marsh species, *Sarcocornia fruticosa* and *Spartina maritima*, significantly accumulated Pb, to maximum concentrations of 2,870 mg Pb/kg and 1,755 mg Pb/kg, respectively (Caetano et al., 2007). Roots had similar isotopic signature to those of sediments in vegetated zones indicating that Pb uptake by plants reflects the input in sediments. BCFs for Pb in root tissue from mangrove tree species range between 0.09 and 2.9, depending on the species and the habitat, with an average BCF of 0.84. The average BCF for mangrove species leaf tissue was considerably less (0.11), as these species are poor translocators of Pb (MacFarlane et al., 2007).

6.4.14.2 Saltwater Invertebrates

Uptake and subsequent bioaccumulation of Pb in marine invertebrates varies greatly between species and across taxa as previously characterized in the 2006 Pb AQCD. This section expands on the findings from the 2006 Pb AQCD on bioaccumulation and sequestration of Pb in saltwater invertebrates. In the case of invertebrates, Pb can be bioaccumulated from multiple sources, including the water column, sediment, and dietary exposures, and factors such as proportion of bioavailable Pb, lifestage, age, and metabolism can alter the accumulation rate. In this section, new information on Pb uptake and subsequent tissue and subcellular distribution will be considered, followed by a discussion on dietary and water routes of exposure and strategies for detoxification of Pb in marine invertebrates.

In marine invertebrates, sites for Pb accumulation include the gill and digestive gland/hepatopancreas. The gills were the main sites of Pb accumulation in pearl oyster, *Pinctada fucata* followed by mantle, in 72-hour exposures to 103.5 µg Pb/L (Jing et al., 2007). Following a 10-day exposure to 2,500 µg Pb/L as Pb nitrate, accumulation of Pb was higher in gill than digestive gland of *Mytilus edulis*: after a 10 day depuration, Pb content was decreased in the gills and digestive gland of these mussels (Einsporn et al., 2009). In blue crabs, *Callinectes sapidus*, collected from a contaminated and a clean

estuary in New Jersey, U.S., the hepatopancreas was found to be the primary organ for Pb uptake (Reichmuth et al., 2010).

There is more information now on the cellular and subcellular distribution of Pb in invertebrates than there was at the time of writing the 2006 Pb AQCD. Specifically, localization of Pb at the ultrastructural level has been assessed in the marine mussel (*M. edulis*) through an antibody-based detection method (Einsporn et al., 2009; Einsporn and Koehler, 2008). Dissolved Pb was detected mainly within specific lysosomal structures in gill epithelial cells and digestive gland cells and was also localized in nuclei and mitochondria. Transport of Pb is thought to be via lysosomal granules associated with hemocytes (Einsporn et al., 2009). In the digestive gland of the variegated scallop *(Chlamys varia*), Pb was also mainly bound to organelles, (66% of the total metal burden) (Bustamante and Miramand, 2005). In the digestive gland of the cephalopod *Sephia officinalis*, (cuttlefish) most of the Pb was found in the organelles (62%) (Bustamante et al., 2006). In contrast, only 7% of Pb in the digestive gland of the octopus (*Octopus vulgaris*) was associated with the fraction containing nuclei, mitochondria, lysosome and microsomes: the majority of Pb in this species was found in cytosolic proteins (Raimundo et al., 2008).

Metian et al. (2009) investigated the uptake and bioaccumulation of ^{210}Pb in variegated scallop and king scallop to determine the major accumulation route (seawater or food) and then assess subsequent tissue distribution. Dietary Pb from phytoplankton in the diet was poorly assimilated (<20%) while more than 70% of Pb in seawater was retained in the tissues. In seawater, ^{210}Pb was accumulated more rapidly in variegated scallop than king scallop and soft tissue distribution patterns differed between the species. Variegated scallop accumulated Pb preferentially in the digestive gland (50%) while in king scallop, Pb was equally distributed in the digestive gland, kidneys, gills, gonad, mantle, intestine, and adductor muscle with each tissue representing 12-30% of ^{210}Pb body load. An additional test with Pb-spiked sediment in king scallop showed low bioaccumulation efficiency of Pb from spiked sediment.

Recently, several studies have attempted to establish biodynamic exposure assessments for various contaminants. In an in situ metal kinetics field study with the mussel *M. galloprovincialis*, simultaneous measurements of metal concentrations in water and suspended particles with mussel biometrics and physiological indices were conducted to establish uptake and excretion rates in the natural environment (Casas et al., 2008). The mean logarithmic ratio of metal concentration in mussels (ng/kg of wet-flesh weight) to metal concentration in water (ng/L) was found to be 4.3 in *M. galloprovincialis*, based on the rate constants of uptake and efflux in a series of transplantation experiments between

contaminated and clean environments. Equilibrium concentrations of Pb in mussels leveled out at approximately 30 days with a concentration of 6.7 mg Pb/kg.

The protective barrier against Pb toxicity formed by the egg structure in some invertebrates was recognized in the 2006 Pb AQCD. Consideration of toxicity of Pb to embryos that develop surrounded by a protective egg shell has been expanded since the 2006 Pb AQCD. In a study with cuttlefish (*S. officinalis*) eggs, radioisotopes were used to assess the permeability of the egg to Pb at low exposure concentrations (^{210}Pb activity concentration corresponding to 512 µg/L Pb) (Lacoue-Labarthe et al., 2009). Retention and diffusion properties of the cuttlefish egg change throughout the development of the embryo and since the eggs are fixed on substrata in shallow coastal waters they may be subject to both acute and chronic Pb exposures. In the radiotracer experiments, ^{210}Pb was never detected in the internal compartments of the egg during the embryonic development stage, while concentrations in the eggshell increased throughout the 48-day exposure. These results are consistent with a study of cuttlefish eggs collected from natural environments in which Pb was only detected in the eggshell. These studies indicate that the cuttlefish egg provides a protective barrier from Pb toxicity (Miramand et al., 2006).

Aquatic invertebrate strategies for detoxifying Pb were reviewed in the 2006 Pb AQCD and include sequestration of Pb in lysosomal-vacuolar systems, excretion of Pb by some organisms, and deposition of Pb to molted exoskeleton. Molting of the exoskeleton can result in depuration of Pb from the body (see Knowlton et al. (1983) and Anderson et al. (1997), as cited in the 2006 Pb AQCD). New research has provided further evidence of depuration of Pb via molting in invertebrates. Mohapatra et al. (2009) observed that Pb concentrations in body tissues were lower in the newly molted mud crabs (*Scylla serrata*) than in the pre-molt, hard-shelled crabs. However, the carapace of hard shelled crabs had lower concentrations of Pb than the exuvium of the soft shell crabs, leading the authors to speculate that some of the metal might be partially excreted during the molting process, rather than entirely through shedding of the previous exoskeleton. Bergey and Weis (2007) showed that differences in the proportion of Pb stored in exoskeleton and soft tissues changed during intermolt and immediate postmolt in two populations of fiddler crabs (*Uca pugnax*) collected from New Jersey. One population from a relatively clean estuary eliminated an average of 56% of Pb total body burden during molting while individuals from a site contaminated by metals eliminated an average of 76% of total Pb body burden via this route. Pb distribution within the body of crabs from the clean site shifted from exoskeleton to soft tissues prior to molting. The authors observed the opposite pattern of Pb distribution in fiddlers from the contaminated site where larger amounts of Pb were depurated in the exoskeleton. The exact dynamics of Pb depuration through molting in crabs are thus still not completely characterized.

6.4.14.3 Saltwater Vertebrates

Saltwater Fish

In comparison to freshwater fish, fewer studies have been conducted on Pb uptake in marine fish. Since marine fish drink seawater to maintain osmotic homeostasis, Pb can be taken up via both gills and intestine (Wang and Rainbow, 2008). Pb was significantly accumulated in gill, liver, plasma, kidney, rectal gland, intestine, skin, and muscle of the elasmobranch spotted dogfish (*Scyliorhinus canicula*) exposed to 2,072 µg Pb/L for one week (De Boeck et al., 2010). In contrast to Pb distribution patterns in freshwater teleosts, high Pb concentrations were present in this species in the skin and rectal gland. Egg cases of the spotted dogfish exposed to ^{210}Pb in seawater for 21 days, accumulated radiolabeled Pb rapidly and the metal was subsequently detected in embryos indicating the permeability of shark eggs to Pb in coastal environments (Jeffree et al., 2008). A study of Pb bioaccumulation in five marine fish species (*Chloroscombrus chrysurus*, *Sardinella aurita*, *Ilisha africana*, *Galeoides decadactylus*, *Caranx latus*) found that *C. chrysurus* was an especially strong bioaccumulator, yielding Pb concentrations of 6 to 10 mg Pb/kg (Gnandi et al., 2006). However, *C. chrysurus* metal content was not correlated to the Pb concentrations along the mine tailings gradient from which they were collected (8.5 and 9.0 µg Pb/L for minimum and maximum tissue concentrations, respectively). This lack of correlation was also observed for fish species that were considered to be weaker Pb bioaccumulators, indicating that unidentified sources of Pb (e.g., in sediments or in dietary sources) may be contributing to Pb uptake by marine fish.

In grunt fish *H. scudderi*, exposed to Pb via dietary uptake through a simulated marine food chain, mean total Pb body burden increased from 0.55 to 3.32 mg Pb/kg in a 42-day feeding study (Soto-Jiménez et al., 2011b). Pb was accumulated to the highest relative concentration in liver with less than 3% of total Pb accumulated in gills. Most of the Pb based on total body mass was accumulated in skeleton, skin, scales and muscle.

The 2006 Pb AQCD considered detoxification mechanisms in fish including mucus production and Pb removal by shedding of scales in which Pb is chelated with keratin. Since the 2006 Pb AQCD review, additional Pb detoxification mechanisms in marine fish have been further elucidated. Mummichog (*Fundulus heteroclitus*) populations in metal-polluted salt marshes in New York exhibited different patterns of intracellular partitioning of Pb although body burden between sites was not significantly different (Goto and Wallace, 2010). Mummichogs at more polluted sites stored a higher amount of Pb in metal rich granules as compared to other detoxifying cellular components such as heat-stable proteins, heat-denaturable proteins and organelles.

Marine Mammals

Studies that consider uptake of Pb in aquatic mammals are limited. Kannan et al. (2006) compared trace element concentrations in livers of free-ranging sea otters (*Enhydra lutris nereis*) found dead along the California coast. They detected Pb in all individuals sampled (N=80) in a range of 0.019 to 1.06 mg Pb/kg. The otters were classified by cause of death (infectious causes, non-infectious causes, emaciated condition) and trace element patterns of tissue distribution were compared. Livers from emaciated otters had significantly elevated levels of Pb compared to non-diseased individuals.

6.4.14.4 Marine Food Web

As discussed in Section 6.4.4.4 trophic transfer of Pb through aquatic food chains was considered to be negligible in the 2006 Pb AQCD (U.S. EPA, 2006c). Measured concentrations of Pb in the tissues of aquatic organisms were found to be generally higher in algae and benthic organisms and lower in higher trophic-level consumers, indicating that Pb was bioaccumulated but not biomagnified (U.S. EPA, 2006c; Eisler, 2000). Recent literature since the 2006 Pb AQCD, provides evidence of the potential for Pb to be transferred in marine food webs while other studies indicate Pb is decreased with increasing trophic level. This section incorporates recent literature on transfer of Pb through marine food chains.

In a dietary study using environmentally realistic concentrations of Pb in prey through four levels of a simplified marine food chain, biological responses including decreased growth and survival and changes in behavior were observed at different trophic levels. However, the concentration of Pb did not increase along the trophic gradient (Soto-Jiménez et al., 2011b; Soto-Jiménez et al., 2011a). The base of the simulated food chain was the microalgae *Tetraselmis suecica* (phytoplankton) grown in 20 µg Pb/L. Pb-exposed cultures of *T. suecica* had significantly less cell divisions per day (growth), biomass and total cell concentrations than control microalgae at 72 hours of exposure. The microalgal cultures were then fed to *Artemia franciscana* (crustacean, brine shrimp) which were then fed to *Litopenaeus vannamei* (crustacean, whiteleg shrimp) and finally to *Haemulon scudderi* (fish, grunt). Effects on behavior, growth and survival were observed in shrimp and in grunt fish occupying the intermediate and top levels of the simulated marine food chain. The authors speculate that the species used in the simulated food chain were able to regulate and eliminate Pb (Soto-Jiménez et al., 2011b).

Partial evidence for biomagnification was observed in a subtropical lagoon in Mexico with increases of Pb concentration occurring in 14 of the 31 (45.2%) of trophic interactions considered (Ruelas-Inzunza and Páez-Osuna, 2008). The highest rate of

transference of Pb as measured in muscle tissue occurred between the prey species whiteleg shrimp (*Litopenaeus vannamei*) and mullet (*Mugil cephalus*) to pelican (*Pelecanus occidentalis*).

Other studies have traced Pb in marine food webs and have found no evidence of biomagnification of Pb with increasing trophic level. In the southeastern Gulf of California, Mexico, Pb was not positively transferred (biomagnification factor <1) through primary producers (seston, detritus) and 14 consumer species in a lagoon food web (Jara-Marini et al., 2009). In a planktonic food web in Bahía Blanca estuary, Argentina, Pb levels in macrozooplankton and mesozooplankton exhibited temporal fluctuations, however no biomagnification was observed between mesozooplankton and macrozooplankton (Fernández Severini et al., 2011). It is important to note, however, that even in the absence of biomagnification, aquatic organisms can bioaccumulate relatively large amounts of metals and become a significant source of dietary metal to their predators (Fairbrother et al., 2007; Reinfelder et al., 1998).

6.4.15 Biological Effects of Pb in Saltwater Systems

This section focuses on the studies of biological effects of Pb on marine and estuarine algae, plants, invertebrates, fish and mammals published since the 2006 Pb AQCD. Key studies from the 1977 Pb AQCD, the 1986 Pb AQCD and the 2006 Pb AQCD on biological effects of Pb are summarized where appropriate. Biological effects of Pb on saltwater algae and plant species are considered below, followed by information on effects on marine invertebrates and vertebrates. Alterations to reproduction, growth and survival of saltwater organisms can lead to changes at the community and ecosystem levels of biological organization such as decreased abundance, reduced taxa richness, and shifts in species composition (Section 6.1). New evidence for Pb effects on reproduction, growth and survival in saltwater plants, invertebrates and vertebrates is summarized in Table 6-6. In general, Pb toxicity to saltwater organisms is less well characterized than toxicity of Pb in freshwater ecosystems due to the fewer number of available studies on marine species. Because this review is focused on effects of Pb, studies reviewed for this section include only those for which Pb was the only, or primary, metal to which the organism was exposed. All reported values are from exposures in which concentrations of Pb were analytically verified unless nominal concentrations are stated.

6.4.15.1 Saltwater Algae and Plants

New evidence on toxicity of Pb to marine algae indicates that species exhibit varying sensitivities to Pb in saltwater. The lowest 72-hour EC_{50} for growth inhibition reported for marine algae was 105 µg Pb/L in *Chaetoceros* sp (Debelius et al., 2009). The microalgae *T. suecica*, had statistically significant decreased biomass yield, growth rate and cell count following 72 hours nominal exposure to 20 µg Pb/L (Soto-Jiménez et al., 2011b). Pb tested at nominal concentrations up to ~2,000 µg Pb/L over a 14-day period did not affect photosynthetic activity in seven species of marine macroalgae (*Ascophyllum nodosum, Fucus vesiculosus, Ulva intestinalis, Cladophora rupestris, Chondrus crispus, Palmaria palmate, Polysiphonia lanosa*) as measured by pulse amplitude modulation chlorophyll fluorescence yield although Pb was readily accumulated by these species (Baumann et al., 2009). In a recent review of the production of phytochelatins and glutathione by marine phytoplankton in response to metal stress, Kawakami et al. (2006) included several studies in which Pb exposure was shown to induce glutathione and phytochelatin at high concentrations in a few species.

6.4.15.2 Saltwater Invertebrates

No studies with marine invertebrates were reviewed in the 1977 Pb AQCD or the 1986 Pb AQCD. Effects of Pb on marine invertebrates reported in the 2006 Pb AQCD included impacts on embryo development in bivalves with an EC_{50} of 221 µg Pb/L for embryogenesis, gender differences in sensitivity to Pb in copepods and increasing toxicity with decreasing salinity in mysids. Survival, growth, and reproduction are affected by Pb in marine organisms. Pb has also been shown to affect stress responses, antioxidant activity, and osmoregulation.

Recent literature strengthens the evidence indicating that Pb affects enzymes and antioxidant activity in marine invertebrates. Most of these studies only report nominal concentrations of Pb. Activity of enzymes associated with the immune defense system in the mantle of pearl oyster were measured at 0, 24, 48 and 72 hour nominal exposure to 100 µg Pb/L (Jing et al., 2007). Activity of AcPase, a lysosomal marker enzyme, was detected at 24 hours and subsequently decreased. Phenoloxidase activity was depressed compared with controls and remained significantly lower than control after 72 hours of exposure to Pb. Increased SOD activity was observed in the mantle but decreased with time, although always remaining higher than in the control animals (Jing et al., 2007). Activity of Se-dependent glutathione peroxidase did not change with Pb exposure. SOD, catalase, and glutathione peroxidase were significantly reduced at environmentally relevant concentrations of Pb (2 µg Pb/L as measured in Bohai Bay, China) in the

digestive gland of the bivalve *Chlamys farreri (*Zhang et al., 2010b*)*. In contrast, Einsporn et al. (2009) observed no change in catalase activity in the digestive gland and gill of blue mussel *M. edulis* following nominal exposure to 2,500 µg Pb/L as Pb nitrate for 10 days and again following a 10 day depuration period. However, in this same species, glutathione-S-transferase activity was elevated in the gills after Pb exposure and remained active during depuration while no changes to glutathione-S-transferase activity were observed in the digestive gland. In black mussel (*M. galloprovincialis*) exposed 10 days to sublethal nominal concentrations of Pb, fluctuations in SOD activity were observed over the length of the exposure and MDA levels were increased in mantle and gill (Vlahogianni and Valavanidis, 2007). Catalase activity was decreased in the mantle of these mussels but fluctuated in their gills, as compared with the control group. In the bivalve *C. farreri* exposed to Pb, there was induction of lipid peroxidation measured as MDA of 24% and a 37% reduction in 7-ethoxyresorufin-o-deethylase (EROD) activity when compared to controls (Zhang et al., 2010b). In red fingered marsh crab, *Parasesarma erythrodactyla*, collected from sites along an estuarine lake in New South Wales, Australia, elevated glutathione peroxidase activity was correlated with individuals with higher metal body burdens (MacFarlane et al., 2006).

ALAD is a recognized biomarker of exposure across a wide range of taxa including bacteria (Korcan et al., 2007), invertebrates and vertebrates. Since the 2006 Pb AQCD, there are additional studies measuring changes in ALAD activity in field-collected bivalves and crustaceans from saltwater habitats. In the bivalve *Chamelea gallina* collected from the coast of Spain, ALAD inhibition was greater with higher concentrations of Pb measured in whole tissue (Kalman et al., 2008). In another study conducted in Spain, ALAD activity was negatively correlated with total Pb concentration in seven marine bivalves (*C. gallina*, *Mactra corallina*, *Donax trunculus*, *Cerastoderma edule*, *M. galloprovincialis*, *Scrobicularia plana* and *Crassostrea angulata*). However, the authors of this study indicated the need to consider variability of responses between species when using ALAD as a biomarker for Pb (Company et al., 2011). Pb content varied significantly among species and was related to habitat (sediment versus substrate) and feeding behavior.

Behavioral responses of aquatic invertebrates to Pb reviewed in the 2006 Pb AQCD included avoidance. A limited number of recent studies have considered additional behavioral endpoints in marine organisms. Valve closing speed was used as a measure of physiological alterations due to Pb exposure in the Catarina scallop (Sobrino-Figueroa and Caceres-Martinez, 2009). The average valve closing time increased from under one second in the control group to 3 to 12 seconds in juvenile scallops exposed to analytically verified concentrations of Pb as Pb nitrate (40 µg/L to 400 µg/L) for 20 days. Damage to

sensory cilia of the mantle was observed following microscopic examination of Pb-exposed individuals.

Since the 2006 Pb AQCD, limited studies on marine invertebrates have indicated effects of Pb on reproduction. In a long term (approximately 60 days) sediment multigenerational bioassay with the estuarine-sediment dwelling amphipod *Elasmopus laevis*, onset to reproduction was significantly delayed at 118 mg Pb/kg compared to controls. In the higher concentrations, start of offspring production was delayed further; 4 days in 234 mg Pb/kg and 8 days in 424 mg Pb/kg (Ringenary et al., 2007). Fecundity and time of first offspring production was also reduced with increasing Pb concentration in sediment above 118 mg Pb/kg. The authors indicate that this concentration is below the current marine sediment regulatory guideline for Pb (218 mg Pb/kg sediment) (NOAA, 1999) and that reproductive endpoints are more sensitive than survival in this species. Exposure of gametes to Pb prior to fertilization resulted in a decrease of the fertilization rates of the marine polychaete *Hydroides elegans* (Gopalakrishnan et al., 2008). In sperm pretreated in 97 µg Pb/L filtered seawater for 20 minutes, fertilization rate decreased by approximately 70% compared to controls. In a separate experiment, eggs were pretreated with Pb prior to addition of an untreated sperm suspension. The fertilization rate of eggs pretreated in 48 µg Pb/L filtered seawater decreased to 20% of the control. In another test with *H. elegans* in which gametes were not pre-treated, but instead added directly to varying concentrations of Pb for fertilization, there appears to be a protective effect following fertilization due to the formation of the fertilization membrane during the first cell division that may prevent Pb from entering the oocytes (Gopalakrishnan et al., 2007).

As noted in the 2006 Pb AQCD and supported by recent studies, Pb exposure negatively affects the growth of marine invertebrates. Wang et al., (2009d) observed growth of embryos of the Asian Clam (*Meretrix meretrix*) was significantly reduced by Pb with an EC_{50} of 197 µg/L. In juvenile Catarina scallop, *Argopecten ventricosus*, exposed to Pb for 30 days, the EC_{50} for growth was 4,210 µg Pb/L (Sobrino-Figueroa et al., 2007). Rate of growth of the deposit feeding *Capitella* sp. polychaetes decreased significantly from the controls in 3 and 6-day exposures, however, the observed changes did not exhibit a clear dose response with increasing Pb concentration (Horng et al., 2009).

Although Pb is known to cause mortality when invertebrates are exposed to sufficiently high concentrations, some species may not exhibit significant mortality even at high concentrations. In a 10-day Pb-spiked sediment exposure (1,000 mg Pb/kg and 15 µg Pb/L dissolved Pb in pore water), 100% of individuals of the Australian estuarine bivalve *Tellina deltoidalis* survived (King et al., 2010). In the deposit feeding *Capitella* sp., polychaetes, exposure to varying concentrations of Pb associated with spiked

sediment up to 870 mg Pb/kg had no effect on survival (Horng et al., 2009). No differences in adult survival were observed in 28 and 60 day sediment exposures to a range of Pb concentrations from 58 mg Pb/kg to 424 mg Pb/kg in the amphipod *E. laevis* (Ringenary et al., 2007). Other species are more sensitive to Pb and these responses are reviewed in Section 6.4.16.

6.4.15.3 Saltwater Vertebrates

Saltwater Fish

There is a dearth of information in previous Pb AQCDs on Pb effects in saltwater fish. Recent data available since the 2006 Pb AQCD include a study with a marine elasmobranch. De Boeck et al. (2010) exposed the spotted dogfish (*S. canicula*) to 2,072 µg Pb/L for one week and measured metallothionein induction in gill and liver tissue, and the electrolytes Na, K, Ca^{2+} and Cl, in plasma. No effects were observed in Pb-exposed fish for any of the physiological variables measured in this study, although Pb was detected in all organs (De Boeck et al., 2010).

Since the 2006 Pb AQCD, several studies integrating behavioral and physiological measures of Pb toxicity have been conducted on marine fish. The ornate wrasse (*Thalassoma pavo*) was exposed nominally to sublethal (400 µg Pb/L) or a maximum acceptable toxicant concentration (1,600 µg Pb/L) dissolved in seawater for one week to assess the effects of Pb on feeding and motor activities (Giusi et al., 2008). In the sublethal concentration group, hyperactivity was elevated 36% over controls. In the high concentration, a 70% increase in hyperactivity was observed and hyperventilation occurred in 56% of behavioral observations. Elevated expression of heat shock protein 70/90 orthologs was detected in the hypothalamus and mesencephalic areas of the brains of Pb-treated fish and neuronal damage was observed in the posterior hypothalamic area and optic tectum. No changes in feeding activity were noted between non-treated and treated fish.

Additional behavioral studies in fish consider effects of dietary Pb. The grunt fish *H. scudderi*, occupying the top level of a simulated marine food chain, exhibited lethargy and decreased food intake during the last week of a 42-day feeding study (Soto-Jiménez et al., 2011b). The fish were fed white shrimp exposed to Pb via brine shrimp that were in turn fed microalgae cultured at a nominal concentration of 20 µg Pb/L. Pb was quantified in shrimp and fish. The authors noted a few of the fish exposed to Pb via dietary transfer through the food chain were observed surfacing and speculated that this behavior was air breathing as a response to stress.

Evidence for reproductive effects of Pb in saltwater fish is limited to a field study in which decreased oocyte diameter and density in the toadfish (*Tetractenos glaber*) were associated with elevated levels of Pb in the gonad of fish collected from contaminated estuaries in Sydney, Australia (Alquezar et al., 2006). The authors state this is suggestive of a reduction in egg size which ultimately may lead to a decline in female reproductive output.

Mammals

Although Pb continues to be detected in tissues of marine mammals in U.S. coastal waters (Bryan et al., 2007; Stavros et al., 2007; Kannan et al., 2006) few studies exist that consider biological effects associated with Pb exposure. Pb effects on immune variables, including cell viability, apoptosis, lymphocyte proliferation, and phagocytosis were tested in vitro on phagocytes and lymphocytes isolated from the peripheral blood of bottlenose dolphin (*Tursiops truncates*) (Cámara Pellissó et al., 2008). No effects on viability of immune cells, apoptosis, or phagocytosis were observed in 72-hour exposure to nominal concentrations of 1,000, 10,000, 20,000 and 50,000 µg Pb/L. Proliferative response of bottlenose dolphin leukocytes was significantly reduced at 50,000 µg Pb/L, albeit by only 10% in comparison to the control. This in vitro exposure with nominal concentrations of Pb is likely not relevant for assessing effects of atmospherically-deposited Pb on marine mammals, however, no additional studies were available for review on the effects on Pb on these organisms.

6.4.16 Exposure and Response of Saltwater Species

Evidence regarding exposure-response relationships and potential thresholds for Pb effects on saltwater populations can inform determination of standard levels that are protective of marine ecosystems. The Annex of the 2006 Pb AQCD (U.S. EPA, 2006c) summarized data on exposure-response functions for invertebrates (Table AX7-2.4.1) (Table AX7-2.4.2). The recent exposure-response studies reviewed in this section expand on earlier findings with information on microalgal and invertebrate species. Studies specific to growth, reproduction and survival endpoints are summarized in Table 6-6. All reported values are from exposures in which concentrations of Pb were analytically verified unless nominal concentrations are stated.

A series of 72-hour Pb toxicity tests were conducted with five marine microalgae species (*T. chuii, R. salina*, Chaetoceros sp., *I. galbana* and *N. gaditana*) to determine the relative Pb sensitivities as measured by growth inhibition. The respective 72-hour EC_{50} values derived were 2,640, 900, 105, 1,340, and 740 µg Pb/L (Debelius et al., 2009). The

authors noted that species cellular size, sorption capacity, or taxonomy did not explain differences in sensitivity to Pb, leaving the mechanism of response still open to question.

In the deposit feeding polychaete, *Capitella* sp. an LOAEL of 85 mg Pb/kg sediment was established in 3 day and 6 day growth experiments (Horng et al., 2009). Other studies of marine invertebrates published since the 2006 Pb AQCD (U.S. EPA, 2006c) have indicated differences in sensitivity of different lifestages of aquatic organisms to Pb. In a series of seawater and sediment exposures using adult and juvenile amphipods *Melita plumulosa*, juveniles were more sensitive to Pb than adults (King et al., 2006). In the seawater-only exposures, the 96-hour LC_{50} was 1,520 µg Pb/L for juveniles and 3,000 µg Pb/L for adults. In comparison,10 day juvenile sediment test results were LC_{50} 1,980, NOEC 580 and LOEC 1,020 mg Pb/kg dry weight compared to the LC_{50}, NOEC, and LOEC value for the adults exposed in sediment (3,560 mg Pb/kg dry weight). A 24-hour LC_{50} of 4,500 µg Pb/L for adult black mussel (*M. galloprovincialis*) suggests that, in general, juvenile bivalves are more sensitive to Pb exposure than adults although this value was based on nominal exposure data (Vlahogianni and Valavanidis, 2007).

Since the 2006 Pb AQCD, Pb toxicity to larval stages of marine species has been assessed at sublethal and lethal concentrations. The effective concentrations at which Pb resulted in 50% of abnormal embryogenesis of the Asian clam (*M. meretrix*) was 297 µg Pb/L. The 96-hour LC_{50} for larvae of the same species was 353 µg Pb/L (Wang et al., 2009d). In comparison, juvenile Catarina scallop (*A. ventricosus*) had a LC_{50} of 830 µg Pb/L in a 96-hour exposure (Sobrino-Figueroa et al., 2007). In the marine polychaete *H. elegans*, EC_{50} values of gametes, embryos, larvae (blastula to trochophore and larval settlement), and adults, exhibited dose-responses to Pb that reflected the differential sensitivity of various lifestages of this organism (Gopalakrishnan et al., 2008). The EC_{50} values for sperm and egg toxicity were 380 and 690 µg Pb/L respectively. Larval settlement measured as the metal concentration causing 50% reduction in attachment was most sensitive to Pb with an EC_{50} of 100 µg Pb/L, while the EC_{50} for abnormal development of embryos was 1,130 µg Pb/L. The LC_{50} values for adult worms in 24-hour and 96-hour tests were 25,017 and 946 µg Pb/L, respectively. Manzo et al. (2010) established a LOEC of 500 µg Pb/L and a maximum effect at 3,000 µg Pb/L in an embryotoxicity assay with sea urchin *P. lividus* exposed to nominal concentrations of Pb. The EC_{50} for developmental defects in this species was 1,250 µg Pb/L with a NOEL of 250 µg Pb/L. In a study using nominal concentrations of Pb, morphological deformities were observed in 50% of veliger larvae of blacklip abalone (*Haliotis rubra*) at 4,100 µg Pb/L following a 48-hour exposure, suggesting this species is not as sensitive to Pb as other marine invertebrate larvae (Gorski and Nugegoda, 2006).

6.4.17 Community and Ecosystem Effects in Saltwater Systems

As discussed in the 1986 Pb AQCD and the 2006 Pb AQCD (U.S. EPA, 2006c), exposure to Pb is likely to have impacts in aquatic environments via effects at several levels of ecological organization (organisms, populations, communities, or ecosystems). But fewer studies explicitly consider community and ecosystem-level effects in marine and brackish waters than in freshwater. Reduced species abundance and biodiversity of protozoan and meiofauna communities were observed in laboratory microcosm studies with marine water and marine sediments reviewed in the 2006 Pb AQCD as summarized in Table AX7-2.5.2 (U.S. EPA, 2006c). In a laboratory study with larval mummichogs reviewed in the 2006 Pb AQCD, feeding and predator avoidance behaviors were altered in this marine fish species following a 4-week exposure to Pb. Observations from field studies reviewed in the 2006 Pb AQCD included findings of a negative correlation between Pb and species richness and diversity indices of macroinvertebrates associated with estuary sediments and changes in species distribution and abundance in fish, crustaceans and macroinvertebrates correlated with Pb levels in marine sediments. The 2006 Pb AQCD concluded that, in general, information from controlled studies for single pollutants was insufficient to permit evaluation of specific impacts on higher levels of organization (beyond the organism). In studies from natural saltwater ecosystems, Pb rarely occurs as a sole contaminant making its effects difficult to ascertain. New information on effects of Pb at the population, community and ecosystem level in coastal ecosystems is reviewed below.

The faunal composition of seagrass beds in a Spanish coastal saltwater lagoon was found to be impacted by Pb in sediment, plants, and biofilm (Marín-Guirao et al., 2005). Sediment Pb concentrations ranged from approximately 100 to 5,000 mg Pb/kg and corresponding biofilm concentrations were 500 to 1,600 mg Pb/kg, with leaf concentrations up to 300 mg Pb/kg. Although multiple community indices (abundance, Shannon-Wiener diversity, Simpson dominance index) did not vary from site to site, multivariate analysis and similarity analysis indicated significant differences in macroinvertebrate communities between sites with different sediment, biofilm, and leaf Pb concentrations. Differences were largely attributable to three amphipod species (*Microdeutopus* sp., *Siphonoecetes sabatieri*, *Gammarus* sp.). This indicates that, although seagrass abundance and biomass were unaffected by Pb exposure, organisms inhabiting these plants still may be adversely impacted.

Caetano et al. (2007) investigated the mobility of Pb in salt marshes using total content and stable isotope signature. They found that roots had similar isotopic signature to sediments in vegetated zones indicating that Pb uptake by plants reflects the input in sediments. At one site, there was a high anthropogenic Pb content while at the other

natural mineralogical sources dominated. The roots of *S. fruticosa* and *S. maritima* significantly accumulated Pb, having maximum concentrations of 2,870 mg Pb/kg and 1,755 mg Pb/kg, respectively, indicating that below-ground biomass played an important role in the biogeochemical cycling of Pb.

Exposure to three levels of sediment Pb contamination (322, 1,225, and 1,465 mg Pb/kg dry weight) had variable effects on different species within a marine nematode community (Mahmoudi et al., 2007). Abundance, taxa richness, and species dominance indices were altered at all Pb exposures when compared with unexposed communities. Further, while the species *Oncholaimellus mediterraneus* dominated control communities (14% of total abundance), communities exposed to low and medium Pb concentrations were dominated by *Oncholaimus campylocercoides* (36%) and *Marylynnia stekhoveni* (32%), and *O. campylocercoides* (42%) and *Chromadorina metulata* (14%), respectively. Communities exposed to the highest Pb sediment concentrations were dominated by *Spirinia gerlachi* (41%) and *Hypodontolaimus colesi* (29%). Given this, the authors concluded that exposure to Pb significantly reduced nematode diversity and resulted in profound restructuring of the community structure.

In another laboratory microcosm experiment with nematodes, nematode diversity and community structure was altered with a mean number of 8 genera present in microcosms contaminated with Pb compared to the control with 20 genera. The spiked sediments used in the study were collected from the Swartkop River estuary, South Africa. Pb (3 to 6,710 mg Pb/kg sediment dry weight) was tested alone and in combination with Cu, Fe, and Zn (Gyedu-Ababio and Baird, 2006). The synergistic effect of the four metals on nematode community structure was greater than the individual metals and the effects of Pb could not be distinguished from Cu, Fe and Zn.

6.4.18 Characterization of Sensitivity and Vulnerability in Saltwater Species

Species differences in metabolism, sequestration, and elimination rates have been shown to control relative sensitivity and vulnerability of exposed organisms and effects on survival, reproduction, growth, metabolism, and development. Diet and lifestage at the time of exposure also contribute significantly to the determination of sensitive and vulnerable populations and communities. Further, environmental conditions in addition to those discussed as affecting bioavailability may also alter Pb toxicity. The 2006 Pb AQCD (U.S. EPA, 2006c) reviewed the effects of genetics, age, and body size on Pb toxicity. While genetics appears to be a significant determinant of Pb sensitivity, effects of age and body size are complicated by environmental factors that alter metabolic rates of saltwater organisms. A review of the more recent literature corroborated these findings, and identified seasonal physiological changes and lifestage as other important determinants of differential sensitivity to Pb.

6.4.18.1 Seasonally Affected Physiological Changes

Couture et al. (2010) investigated seasonal and decadal variations in Pb sources to mussels (*M. edulis*) from the French Atlantic shoreline. Pb concentrations in the mussels were 5-66 times higher than the natural background value for the north Atlantic. The $^{206}Pb/^{207}Pb$ signature indicated that the bioaccumulated Pb was anthropogenic in origin. The signature was not, however, the same as that emitted in western Europe, as a result of leaded gasoline combustion, although that was a major emission source to the atmosphere during a large part of the study period (1985-2005). Instead, it was most similar to that of Pb released into the environment from wastewater treatment plants, municipal waste incinerators and industries such as metal refineries and smelters. Thus continental runoff rather than atmospheric deposition was identified as the main source of Pb to the French coastal area. The strong seasonal variations in $^{206}Pb/^{208}Pb$ were used to conclude that resuspension of Pb triggered by high river runoff events was a key factor affecting bioaccumulation of Pb in *M. edulis*.

In another monitoring study, Pearce and Mann (2006) investigated variations in concentrations of trace metals in the U.K. including Pb in the shells of pod razor shell (*Ensis siliqua*). Pb concentration varied from 3.06-36.2 mg Pb/kg and showed a regional relationship to known sources, e.g., former metal mining areas such as Cardigan Bay, Anglesey, and industrial activity in Liverpool Bay. Seasonal variations were also found for Pb in both Cardigan Bay and Liverpool Bay, relating to increased winter fluxes of Pb (and other metals) into the marine environment. In contrast, levels of Pb and other metals

were highest in summer and lowest in winter in oysters *Crassostrea corteziensis* collected from Sonora, Mexico (García-Rico et al., 2010).

Carvalho et al. (2011) quantified ^{210}Pb in *M. galloprovincialis* sampled at coastal locations in Portugal and noted that the apparent seasonal fluctuation in radionuclide concentrations in mussel soft tissues was mostly attributable to changes in physiological condition (i.e., fat content, gonadal development) and not to radionuclide body burden fluctuation. The authors caution that since concentrations of contaminants are dependent upon tissue composition, corrections for mussel physiological condition are need to compare results from different seasons and different locations.

6.4.18.2 Lifestage

Lifestages of the marine polychaete *H. elegans* including embryogenesis, sexual maturation, and offspring development were shown to be differentially affected by Pb exposure. Pb water concentrations of 91 µg Pb/L and greater significantly affected fertilization and embryonic development, but the greatest effects were exhibited by 24-hour-old larvae (Gopalakrishnan et al., 2007). The authors suggested that timing of Pb exposure may have different impacts on marine polychaete populations, if life cycles are offset (Gopalakrishnan et al., 2007). Further, given that the adult lifestage is sedentary, reduction of the mobile early lifestage as a result of Pb exposures may disproportionally affect sessile polychaetes. For instance, larval settlement was significantly reduced at Pb exposures of 48 µg Pb/L and greater (Gopalakrishnan et al., 2008).

6.4.18.3 Species Sensitivity

Both inter- and intra-specific differences in Pb uptake and bioaccumulation may occur in macroinvertebrates of the same functional feeding group. Data from 20 years of monitoring of contaminant levels in filter-feeding mussels of the *Mytilus* species and *Crassostrea virginica* oysters in coastal areas of the U.S. through the National Oceanic and Atmospheric Administration (NOAA) Mussel Watch program indicate that Pb is on average three times higher in mussels than in oysters (Kimbrough et al., 2008). Limpet (Patella sp.) from the Lebanese Coast had Pb BAF values ranging from 2,500 to 6,000 and in the same field study Pb BAF values for a mussel (*Brachidontes variabilis*) ranged from 7,500-8,000 (Nakhle et al., 2006).

There is some indication that molting may comprise an additional sequestration and excretion pathway for aquatic animals exposed to Pb (Soto-Jiménez et al., 2011a; Mohapatra et al., 2009; Tollett et al., 2009; Bergey and Weis, 2007). Crab species

U. pugnax (Bergey and Weis, 2007) and *Scylla serrata (*Mohapatra et al., 2009*)*, and white shrimp *L. vannamei* (Soto-Jiménez et al., 2011a) have been shown to sequester Pb preferentially in exoskeleton tissue, where it is later shed along with other tissue. Consequently, aquatic arthropod species and those species that shed their exoskeleton more frequently may be able to tolerate higher environmental Pb concentrations than non-arthropods or slow-growing molting species, as this pathway allows them to effectively lower Pb body burdens.

Some tolerant species of fish (e.g., mummichog) have the ability to sequester accumulated Pb in metal-rich granules or heat-stable proteins (Goto and Wallace, 2010). Fish with such abilities are more likely to thrive in Pb-contaminated environments than other species.

6.4.19 Ecosystem Services Associated with Saltwater Systems

Pb deposited on the surface of (or taken up by) organisms has the potential to alter the services provided by saltwater biota to humans although the directionality of impacts is not always clear. For example, oysters and mussels provide a service by sequestering Pb. At the same time, the uptake of Pb by these bivalves may result in toxicological effects associated with Pb exposure and decreased value of shellfish as a commodity. At this time, a few publications address Pb impacts on ecosystem services associated with saltwater ecosystems. Pb can affect the ecological effects in each of the four main categories of ecosystem services (Section 6.1.2) as defined by Hassan et al. (2005). These effects are sorted into ecosystem services categories and summarized here:

- Supporting: food for higher trophic levels, biodiversity
- Provisioning: contamination of food by heavy metals, decline in health of fish and other aquatic species
- Regulating: water quality
- Cultural: ecosystem and cultural heritage values related to ecosystem integrity and biodiversity, wildlife and bird watching, fishing

A few recent studies explicitly consider the impact of Pb and other heavy metals on ecosystem services provided by salt marsh (Gedan et al., 2009) and estuaries (Smith et al., 2009b). These systems are natural sinks for metals and other contaminants. Pb can be toxic to salt marsh plant species and decaying plant detritus may result in resuspension of Pb into the aquatic food chain (Gedan et al., 2009). Salt marsh and estuaries provide habitat and breeding areas for both terrestrial and marine wildlife and are locations for bird watching. Using a modeling approach designed to assess the degree of risk of Pb and Hg to wading birds in estuarine habitats in the U.K., the authors found a high probability

that Pb poses an ecologically relevant risk to dunlin, *Calidris alpina (*Smith et al., 2009b*)*. However, the authors noted that a major source of uncertainty in this study was the NOAEL values for Pb.

The impact of Pb on ecological services provided by specific components of aquatic systems has been considered in a limited number of studies. Recent research has suggested that dietary Pb (i.e., Pb adsorbed to sediment, particulate matter, and food) may contribute to exposure and toxicity in primary and secondary order consumers (including humans). Aquatic fauna can take up and bioaccumulate metals. If the bioaccumulating species is a food source, the uptake of metals may make it toxic or more dangerous for people or other wildlife to consume. For example, oysters and mussels bioaccumulate Pb from anthropogenic sources, including atmospheric deposition, and are a food source that is widely consumed by humans and wildlife (Couture et al., 2010). Their capacity to bioaccumulate Pb makes them good bioindicators of environmental contamination and they have been used as monitors of coastal pollutants by the NOAA Mussel Watch program since 1986. Although bioaccumulation may render aquatic fauna toxic to consumers, bioaccumulation is a way to sequester the metals and remove them from waters and soils. Sequestration for this purpose is itself an ecosystem service and has been quantified. For example, the total ecological services value of a constructed intertidal oyster (*Crassostrea* sp.) reef in improving water quality and sequestering metals including Pb was calculated in the Yangtze River estuary to be about $500,000 per year (Quan et al., 2009).

6.4.20 Synthesis of New Evidence for Pb Effects in Saltwater Systems

This synthesis of the effects of Pb on saltwater ecosystems covers information from the publication of the 2006 Pb AQCD (U.S. EPA, 2006c) to present. It is followed in Section 6.4.21 by determinations of causality that take into account evidence dating back to the 1977 Pb AQCD. In general, evidence for toxicity to saltwater organisms is less well characterized than toxicity of Pb in freshwater ecosystems due to the fewer number of available studies on marine species. The studies that are available for marine plants, invertebrates and vertebrates include studies where Pb concentration was analytically verified and those that reported nominal concentrations (Table 6-6). Many of the studies that report nominal concentrations in media are uptake studies that subsequently quantify Pb in tissues; however, measurement of Pb in water or sediment at the beginning of an exposure is desirable when comparing laboratory studies to concentrations of Pb in marine systems. In Section 6.2.3 and Table 6-2, a range of 0.01 to 27 µg Pb/L was reported for saltwater, including estuaries and open ocean, with the higher values associated with sites involving human activity (Sadiq, 1992).

Most studies on marine organisms reviewed in the present document included concentrations that were higher than Pb encountered in seawater. However, when multiple concentrations were used, effects generally increased with increasing Pb exposure. Effects at lower concentrations can be implied from many reported studies since an exposure response relationship to Pb was observed. In marine and estuarine systems, exposure to Pb from air is most likely characterized as a chronic low dose exposure, however, most studies only report an acute LC_{50} value when an LC_{10} would be a more appropriate measurement for consideration of effects on organisms since an effect occurring at the LC_{50} value would most likely not maintain a stable population.

Plants

Only a few studies were available since the 2006 Pb AQCD that consider effects of Pb on marine algae (Section 6.4.15.1). A 72-hour EC_{50} for growth inhibition was reported in the marine algae *Chaetoceros* sp. at 105 µg Pb/L (Debelius et al., 2009). A study with the green alga *T. suecica* reports a statistically significant decease in growth rate, total dry biomass and final cell concentration between control cultures and algae cultured in 20 µg Pb/L (Soto-Jiménez et al., 2011b). Both of these studies suggest growth effects at or near the highest recorded values of Pb in seawater (27 µg Pb/L), however, effects are likely to occur at lower concentrations since only EC_{50} values are reported.

Invertebrates

In saltwater invertebrates (Section 6.4.15.2 and 6.4.16) there are studies that consider Pb-effects on supporting endpoints (stress responses, hematological effects and neurobehavior) as well as studies that assess Pb impacts to reproduction, growth, and survival; endpoints that have the potential to alter population, community and ecosystem—levels of biological organization. Many studies, especially those that consider enzymatic responses to Pb exposure, were conducted with nominal Pb concentrations. Two of these studies; Jing et al. (2007) and Zhang et al. (2010b), considered Pb nominal exposures at 100 µg Pb/L or lower and reported significant decreases in antioxidant enzyme activity. The Zhang et al. (2010b) study observed effects on enzymatic activity at a nominal exposure of 2 µg Pb/L. Although these effects are near reported Pb concentrations in seawater they were not analytically verified.

Other studies that report sub-organismal responses in saltwater organisms have quantified Pb exposure. Field studies with bivalves collected off the coast of Spain correlated ALAD activity with measured levels of Pb in tissue (Company et al., 2011; Kalman et al., 2008). An increase in valve closing time with increasing Pb exposure in the range of 40 to 400 µg Pb/L was observed in the scallop, *A.ventricosus* (Sobrino-Figueroa and

Caceres-Martinez, 2009). Although the concentrations in this study exceed reported levels of Pb in seawater, the lower range is near 27 µg Pb/L reported by Sadiq (1992).

Evidence for effects on reproduction, growth, and survival in marine invertebrates (Table 6-6) are primarily from studies in which Pb in the exposure media was quantified. In the amphipod, *E. laevis*, onset to reproduction was significantly delayed at 118 mg/Pb kg sediment; a concentration that the authors indicate is below the current marine sediment regulatory guideline for Pb (218 mg Pb/kg sediment) (Ringenary et al., 2007; NOAA, 1999). In the same study, no effects of Pb on adult survival in 28 and 60 day sediment exposures were observed. In another study with amphipods, juvenile *M. plumosa* were more sensitive than adults in 10-day sediment exposures with an NOEC of 580 mg Pb/kg dry weight compared to an NOEC of 3,560 mg Pb/kg dry weight for adults (King et al., 2006). Effects of Pb on gametes of the marine polycheate *H. elegans* were observed at 48 µg Pb/L (Gopalakrishnan et al., 2008), a concentration near the upper range of Pb in seawater reported by Sadiq (1992). Specifically, fertilization rate of eggs pretreated with 48 µg Pb/L decreased to 20% of control. Life stages of *H. elegans* varied in their sensitivity to Pb with the most sensitive period being larval settlement with an EC_{50} of 100 µg Pb/L.

There are only a few recent studies that considered effects of Pb on growth of marine invertebrates (Sections 6.4.15.2 and 6.4.16). In the polychaete *Capitella* sp. growth was decreased significantly from controls, however, there was not a clear dose-response relationship between increasing Pb concentrations and observed effects (Horng et al., 2009). The authors reported a LOAEL of 85 mg Pb/kg in the sediment exposure. In the Asian Clam *M. meretrix*, an EC_{50} of 197 µg Pb/L was reported for growth (Wang et al., 2009d). Other marine invertebrate growth effects were observed at much higher Pb concentrations (Table 6-6).

Survival was a less sensitive endpoint in marine invertebrates than reproduction or growth with no effects reported at concentrations typically observed in seawater (Table 6-6). In the amphipod *M. plumulosa* an NOEC of 400 µg Pb/L for juveniles and an NOEC of 850 µg Pb/L was reported for adults in 96-hour seawater only exposures (King et al., 2006). In 10 day sediment tests with the same species, juveniles were also more sensitive than adults. Other concentrations at which survival effects were reported in marine invertebrates also greatly exceeded concentrations of Pb typically found in seawater.

Vertebrates

There is not sufficient new evidence for saltwater vertebrates especially for reproductive, growth and survival endpoints that may have relevance to the population-level of biological organization and higher.

Food Web

Some studies published since the 2006 Pb AQCD (see Section 6.4.14.4) support the potential for Pb to be transferred in saltwater food webs, while other studies have found no evidence for biomagnification.

Ecosystem Level Effects

Evidence for effects at higher levels of biological organization in saltwater habitats is primarily supported by observations in a small number of microcosm and field studies where shifts in community structure are the most commonly observed effects of Pb (Section 6.4.17). Effects on reproduction, growth or survival (summarized in Table 6-6) may lead to effects at the population-level of biological organization and higher.

6.4.21 Causal Determinations for Pb in Saltwater Systems

In the following sections, organism-level effects on reproduction and development, growth and survival are considered first since these endpoints can lead to effects at the population level or above and are important in ecological risk assessment. Neurobehavioral effects are considered next followed by sub-organismal responses (hematological effects, physiological stress) for which Pb has been shown to have an impact in multiple species and across taxa, including humans. Causal determinations for terrestrial, freshwater and saltwater ecological effects are summarized in Table 6-3.

6.4.21.1 Reproductive and Developmental Effects-Saltwater Biota

Reproductive effects of Pb have been reported in a few marine organisms and the majority of the available studies are with invertebrate species. In a study reviewed in the 2006 Pb AQCD (U.S. EPA, 2006c), embryo development in two commercial bivalves *Ruditapes decussatus* and *M. galloprovincialis* was inhibited by Pb (Beiras and Albentosa, 2003). In *R. decussatus* an EC_{50} range of 156 to 312 µg Pb/L and LOEC of 156 µg Pb/L were observed for inhibition of embryonic development while in

M. galloprovincialis the EC_{50} was 221 µg Pb/L and the LOEC was 50 µg Pb/L. Larvae of the mussel *M. edulis* were sensitive to Pb exposure with an EC_{50} of 476 µg Pb/L for abnormal development of embryos following 48-hour exposure to Pb during embryogenesis (Martin et al., 1981). The LOEC for embryogenesis in the marine bivalve *M. galloprovincialis* was 50 µg Pb/L with an EC_{50} for embryogenesis of 221 µg Pb/L (Beiras and Albentosa, 2003).

Recent evidence for reproductive effects of Pb on marine invertebrates is summarized in Table 6-6. In the marine polychaete *H. elegans* an EC_{50} of 261 µg Pb/L was observed for unhatched or abnormal larvae following 20 hour incubation with Pb (Gopalakrishnan et al., 2008). The EC_{50} for the metal concentration causing 5% reduction in larval attachment was 100 µg Pb/L. The EC_{50} values for sperm and egg toxicity were 380 and 692 µg Pb/L, respectively. The EC_{50} for embryogenesis in the clam *M. meretrix* was 297 µg Pb/L (Wang et al., 2009d). In a multigenerational bioassay with the marine amphipod *E. laevis*, statistically significant delays in onset of reproduction (4 to 8 days), sexual maturation and first offspring were observed at concentrations of 188 mg Pb/kg sediment and higher (Ringenary et al., 2007). The authors indicate that this concentration is below the current sediment regulatory guideline for Pb (218 mg Pb/kg sediment) (NOAA, 1999) and that reproductive effects are a more sensitive endpoint than lethality. Although LC_{50} values are typically reported for Pb effects on reproductive endpoints in saltwater invertebrates, a concentration dependent relationship between reproductive impairment and increasing concentration of Pb is reported in most studies. This exposure-response relationship implies that effects on reproduction are occurring at concentrations lower than the LC_{50} value.

Reproductive effects are only characterized in a few species and endpoints for marine systems. The weight of the current evidence for reproductive effects is limited to laboratory-based studies with saltwater invertebrates in which observed effects occur at Pb concentrations that are higher than Pb concentrations encountered in the marine environment. Evidence for reproductive effects of Pb on marine plant species is limited to one study on the red alga (*Champia parvula*) reviewed in the draft *Ambient Aquatic Life Water Quality Criteria for Lead* report (U.S. EPA, 2008b). In one study from a saltwater fish, field-collected smooth toadfish (*T. glaber*) from metal contaminated estuaries in Sydney, Australia had elevated Pb levels in gonad and decreased oocyte diameter and density. Evidence is, therefore, inadequate to conclude that there is a causal relationship for reproductive effects in saltwater plants, and vertebrates. The available studies on marine invertebrates are suggestive that there is a causal relationship between Pb exposure and reproductive effects.

6.4.21.2 Growth Effects-Saltwater Biota

There are few studies that measure growth effects of Pb on marine organisms; available information is limited to marine flora and invertebrates. Growth studies in saltwater plant species are summarized in Table 4 and Table 6 of the draft *Ambient Aquatic Life Water Quality Criteria for Lead* report (U.S. EPA, 2008b) and Table 6-6 of the present document.

Diatoms are among the most sensitive algae; however, growth effects are typically observed at concentrations of Pb higher than the range of values available from saltwater locations [0.01 to 27 µg Pb/L, (Sadiq, 1992)]. In studies available since the draft *Ambient Aquatic Life Water Quality Criteria for Lead* report, the lowest 72-hour EC_{50} for growth inhibition reported in marine diatoms was 105 µg Pb/L in Chaetoceros sp (Debelius et al., 2009) and the growth of the green alga *T. suecica* exposed nominally to 20 µg Pb/L was 40% lower than control cultures (Soto-Jiménez et al., 2011b). The microalgae was the base of a simulated marine food chain including primary, secondary and tertiary level consumers and effects on survival were observed at the higher trophic levels that originated from Pb exposure via consumption of the primary producer. The majority of growth effects reported in saltwater algae exceed concentrations of Pb in seawater by several orders of magnitude. Effects of Pb on growth in two species of brown algae, *Fucus vesiculosus* and *Fucus serratus* are summarized in Table 6 of the draft *Ambient Aquatic Life Water Quality Criteria for Lead* report (U.S. EPA, 2008b). Concentrations where growth impairment was observed in these species greatly exceed available values for Pb measured in seawater.

In saltwater invertebrates, evidence for growth effects is limited to a few species at concentrations that exceed Pb concentrations reported in seawater. Growth inhibition in the bivalve *Macoma balthica* (EC_{50}=453.4 µg Pb/L) is reported in Table 6 of the draft *Ambient Aquatic Life Water Quality Criteria for Lead* report (U.S. EPA, 2008b). Recent studies include Wang et al., (2009d) in which observed growth of embryos of the Asian Clam (*M. meretrix*) was significantly reduced by Pb with an EC_{50} of 197 µg Pb/L. In juvenile Catarina scallop, *A. ventricosus*, exposed to Pb for 30 days, the EC_{50} for growth was 4,210 µg Pb/L (Sobrino-Figueroa et al., 2007). Rate of growth of the deposit feeding polychaete Capitella sp. exposed to Pb-spiked sediments from polluted estuaries decreased significantly from the control; however, changes were inconsistent with increasing concentration of Pb (Horng et al., 2009). Evidence is therefore inadequate to conclude that there is a causal relationship between Pb exposure and growth effects in saltwater plants, invertebrates and vertebrates.

6.4.21.3 Survival-Saltwater Biota

There are no studies reported in the previous Pb AQCDs or the current ISA for aquatic plants that indicate phytotoxicity at or near current concentrations of Pb in saltwater [0.01 to 27 µg Pb/L, (Sadiq, 1992)].

Mortality data for saltwater invertebrate species are summarized in the draft *Ambient Aquatic Life Water Quality Criteria for Lead* report (U.S. EPA, 2008b) and reported LC_{50} values greatly exceed Pb concentrations encountered in seawater. Recent studies available since the 2006 Pb AQCD, and the draft *Ambient Aquatic Life Water Quality Criteria for Lead* document, that report mortality data are summarized in Table 6-6. In general, marine fauna are less sensitive to this metal than freshwater fauna and the highest toxicity is observed in juveniles. A 144-hour LC_{50} of 680 µg Pb/L was reported for juvenile scallop *A. ventricosus* (Sobrino-Figueroa et al., 2007) and a 96-hour LC_{50} of 353 µg Pb/L for embryos of the clam *M. meretrix* (Wang et al., 2009d). In the amphipod *M. plumulosa*, juveniles were more sensitive to Pb than adults in 96 hour seawater-only exposures and 10 day sediment exposures (King et al., 2006). The 96-hour LC_{50} was 1,520 µg Pb/L and the NOEC was 400 µg Pb/L for juveniles in comparison to adults (96-hour LC_{50} =3,000 µg Pb/L; NOEC=1,680 µg Pb/L). In the 10-day sediment exposures, the NOEC for juveniles was 580 mg Pb/kg dry weight compared to an adult NOEC of 3,560 mg Pb/kg dry weight. In 10-day exposures to Pb nitrate spiked sediment, all individuals of the bivalve *T. deltoidalis* survived at 1,000 mg/Pb kg with 15 µg Pb/L dissolved in pore water (King et al., 2010). No effects on survival were observed in either the amphipod *E. laevis* exposed 60 days to Pb-spiked sediment up to 424 mg Pb/kg (Ringenary et al., 2007), or in the polychaete *Capitella* sp. exposed to sediment for 3 or 6 days up to 871 mg Pb/kg (Horng et al., 2009).

Effects of Pb on survival have been demonstrated though a simulated marine food chain in which the primary producer, the microalgae *T. suecica*, was exposed nominally to 20 µg Pb/L and subsequently fed to brine shrimp *A. franciscana*, (mean Pb content 12 to 15 mg Pb/kg) which were consumed by white-leg shrimp *L. vannamei*, itself consumed by grunt fish *H. scudderi* representing the top of the marine food chain (Soto-Jiménez et al., 2011b). Survival of brine shrimp was 25 to 35% lower than the control and both white shrimp and grunt fish had significantly higher mortalities than controls.

Data on Pb toxicity to eight species of marine fishes are summarized in Table 1 of the draft *Ambient Aquatic Life Water Quality Criteria for Lead* report (U.S. EPA, 2008b). All of the LC_{50} values for these fish (range 1,500 to 315,000 µg Pb/L) greatly exceed concentrations of Pb reported in seawater. Additionally, in the 2006 Pb AQCD (U.S. EPA, 2006c) the acute toxicity of Pb to plaice (*Pleuronectes platessa*) was reported to range from 50 µg Pb/L to 300,000 µg Pb/L depending on the form of Pb (Eisler, 2000).

The existing evidence on toxicity of Pb to marine vertebrates is limited to laboratory-based studies conducted under different salinities and exposure conditions. Considerable uncertainties exist in applying laboratory observations to actual conditions in the field where other modulating factors can affect Pb bioavailability and toxicity.

Although evidence exists for increased mortality of marine fish at very high concentrations of Pb, the focus of the causal determinations are on studies where effects were observed within one to two orders of magnitude of Pb measured in the environment (Table II of the Preamble). Evidence is therefore inadequate to conclude that there is a causal relationship between Pb and survival in saltwater plants, invertebrates, and vertebrates.

6.4.21.4 Neurobehavioral Effects-Saltwater Biota

In marine organisms evidence for neurobehavioral effects of Pb is limited to a few studies on bivalves and fish. In a study reviewed in the 2006 Pb AQCD (U.S. EPA, 2006c), prey capture rate and predator avoidance was affected in mummichogs starting at 300 µg Pb/L (Weis and Weis, 1998). Recent studies support previous findings of decreased ability to escape predation associated with Pb exposure. In juvenile Catarina scallops exposed to Pb (40 µg/L to 400 µg/L) for 20 days, the average valve closing time increased from under one second in the control group to 3 to 12 seconds in juvenile scallops A decrease in valve closing speed in these bivalves may impact escape swimming behaviors important for predator avoidance (Sobrino-Figueroa and Caceres-Martinez, 2009). Behavioral effects in grunt fish *H. scudderi*, occupying the top level of a simulated marine food chain included lethargy and decreased food intake in a 42-day feeding study (Soto-Jiménez et al., 2011b). These fish were fed white shrimp exposed to Pb via brine shrimp that were initially fed microalgae cultured at a nominal concentration of 20 µg Pb/L. In the same study, surfacing, reduction of motility, and erratic swimming were observed in the white shrimp after 30 days of exposure to Pb via diet. The ornate wrasse, *T. pavo*, was exposed nominally to sublethal (400 µg Pb/L) or a maximum acceptable toxicant concentration (1,600 µg Pb/L) dissolved in seawater for one week to assess the effects of Pb on feeding and motor activities (Giusi et al., 2008). In the sublethal concentration group, hyperactivity was elevated 36% over controls. In the high concentration, a 70% increase in hyperactivity was observed and hyperventilation occurred in 56% of behavioral observations, however, no changes in feeding activity were noted between non-treated and treated fish.

Most of the evidence for neurobehavioral changes in marine organisms is observed with concentrations of Pb that exceed the range of Pb values available for saltwater of 0.01 to

27 µg Pb/L (Sadiq, 1992)], with the exception of the food chain study discussed above in which behavioral effects were observed in shrimp and their fish predators following ingestion of microalgae cultured in nominal concentration of 20 µg Pb/L and then quantified in prey (Soto-Jiménez et al., 2011b). Marine species are typically underrepresented in toxicity testing of behavioral endpoints with metals. There are considerable uncertainties in applying observations from laboratory-based studies to field scenarios including the role of environmental factors such as salinity and DOM on Pb bioavailability. Evidence is inadequate to conclude that there is a causal relationship between Pb exposures and neurobehavioral endpoints in saltwater invertebrates and vertebrates.

6.4.21.5 Hematological Effects-Saltwater Biota

Evidence for hematological effects of Pb on saltwater organisms is limited primarily to field monitoring studies on bivalves. Several recent field studies using a multi-biomarker approach to study the sources and impacts of Pb in marine environments have measured ALAD activity in bivalve species and found positive correlations between increased tissue Pb levels and ALAD inhibition (e.g., Company et al. (2011), Kalman et al (2008)). Generally, these studies have noted that Pb content varies significantly among species and is related to habitat and feeding behavior. There is precedent, especially in Europe, for the inclusion of ALAD as a biomarker of exposure to Pb in marine invertebrates. The mechanism of ALAD inhibition in response to Pb exposure is likely mediated through a common pathway in both marine and freshwater invertebrates (Section 6.4.12.5) as well as in terrestrial species (Section 6.3.12.5) and humans (Section 4.7). Evidence is therefore, suggestive of a causal relationship between Pb exposure and hematological effects in saltwater invertebrates. Evidence is inadequate to conclude that there is a causal relationship between hematological effects and saltwater vertebrates.

6.4.21.6 Physiological Stress-Saltwater Biota

Most studies on physiological stress responses in marine invertebrates are laboratory-based exposures where effects are observed at Pb concentrations that exceed those known to occur in seawater [0.01 to 27 µg Pb/L (Sadiq, 1992), Table 6-2]. However, some recent evidence for invertebrate antioxidant response in bivalves and crustaceans indicates effects may occur at Pb concentrations that are detected in the marine environment. For example, SOD, catalase, and glutathione peroxidase activities were significantly reduced in the digestive gland of the marine bivalve *C. farreri* at 2 µg Pb/L (as measured in Bohai Bay, China) (Zhang et al., 2010b). In red fingered marsh crabs,

P. erythrodactyla collected from an estuarine lake in Australia, elevated glutathione peroxidase activity was correlated with individuals with higher metal body burdens (MacFarlane et al., 2006).

Additional evidence from environmental monitoring studies that compared biomarker responses between reference and contaminated sites indicated a correlation between the amount of Pb with changes in antioxidant enzyme activity [e.g., (Serafim et al., 2011; Cravo et al., 2009)]. Marine bivalves are the organisms typically sampled for these biomonitoring studies since both metals and enzymatic activities can be readily measured in these invertebrates. Although these studies show clear evidence of alterations in antioxidant stress markers in response to marine pollution, these effects cannot be attributed solely to Pb in the environment due to the presence of other metals and contaminants. Evidence for stress responses in marine organisms is typically limited to invertebrates, however, elevated expression of heat shock protein orthologs were reported for the first time in the hypothalamic and mesencephalic brain regions of Pb-treated fish (Giusi et al., 2008).

Evidence for physiological stress responses in saltwater invertebrates are supported by evidence in freshwater species (Section 6.4.12.6) and terrestrial species (Section 6.3.12.6) as well as in humans and experimental animal studies of oxidative stress following impairment of normal metal ion functions (Section 4.2.4). Stress responses may increase susceptibility to other stressors and reduce individual fitness. Evidence is suggestive of a causal relationship between Pb exposures and physiological stress in saltwater invertebrates. The evidence is inadequate to conclude that there is a causal relationship between Pb exposure and physiological stress in saltwater plants and vertebrates.

6.4.21.7 Community and Ecosystem Level Effects-Saltwater Biota

No studies on community and ecosystem level effects of Pb in marine systems were reviewed in the 1977 Pb AQCD (U.S. EPA, 1977), or the 1986 Pb AQCD (U.S. EPA, 1986a). Observations from field studies reviewed in the 2006 Pb AQCD (U.S. EPA, 2006c) included findings of a negative correlation between Pb and species richness and diversity indices of macroinvertebrates associated with estuary sediments (summarized in Table AX7-2.5.2 of the 2006 Pb AQCD). Additional findings in marine environments included changes in species distribution and abundance in fish, crustaceans and macroinvertebrates correlated with Pb levels in marine sediments.

New evidence for community and ecosystem level effects of Pb in saltwater ecosystems includes laboratory microcosm studies as well as observations from field-collected sediments, biofilm and plants in which changes in community structure were observed. In

a recent study, significant differences in macroinvertebrate communities associated with seagrass beds were reported between sites with different sediment, biofilm, and leaf Pb concentrations (Marín-Guirao et al., 2005). Sediment Pb concentrations ranged from approximately 100 to 5,000 mg Pb/kg and corresponding biofilm concentrations were 500 to 1,600 mg Pb/kg, with leaf concentrations up to 300 mg Pb/kg. In a laboratory microcosm experiment conducted with estuarine sediments from South Africa, total meiofauna density decreased (range 3 to 5 taxa) after 32 days in Pb-treated (1,886 to 6,710 µg/Pb g sediment dry weight) sediments compared to 9 taxa in the control (3 µg/Pb g sediment dry weight) (Gyedu-Ababio and Baird, 2006). In a microcosm experiment, exposure to three levels of sediment Pb contamination (322, 1,225, and 1,465 mg Pb/kg dry weight) significantly reduced marine nematode diversity and resulted in profound restructuring of the community structure (Mahmoudi et al., 2007).

There is not sufficient information at this time to characterize and to quantify relationships between ambient concentrations of Pb and response in saltwater communities and ecosystems. Fewer studies are available for saltwater organisms when compared to freshwater systems. There are likely differences in uptake and bioaccumulation in marine species due to physiological characteristics for adaptation in salt water. Additional uncertainties in evaluating the effects of Pb in marine environments include the presence of multiple stressors, inherent natural variability, and differences in Pb bioavailability across saltwater ecosystems. Evidence is inadequate to establish if there is a causal relationship between Pb exposures and the alteration of species richness, species composition and biodiversity in saltwater ecosystems.

6.5 Causal Determinations for Ecological Effects of Pb

This section summarizes the key conclusions regarding causality for welfare effects of Pb. Causal determinations for reproductive, growth, survival, neurobehavioral, hematological and physiological stress endpoints are presented separately for terrestrial, freshwater, and saltwater organisms (Sections 6.3.12, 6.4.12, and 6.4.21). In Section 1.7.3, causal determinations for the same endpoints are further integrated across terrestrial, freshwater, and saltwater taxa. Evidence considered in establishing causality was drawn from findings presented in the 1977 (U.S. EPA, 1977), 1986 (U.S. EPA, 1986a) and 2006 Pb AQCDs (U.S. EPA, 2006c), integrated with an exhaustive review of more recent evidence. The causal statements for terrestrial, freshwater and saltwater effects are divided into two categories: (1) endpoints that are commonly used in ecological risk assessment (reproduction, growth and survival) because they clearly can lead to population-level (e.g., abundance, production, extirpation), community-level (taxa richness, relative abundance) and ecosystem-level effects (Ankley et al., 2010; Suter et

al., 2005), and (2) organism and sub-organism responses such as neurobehavioral effects, hematological effects and physiological stress. There are many different effects at the molecular and cellular levels, and chronic toxicity of Pb in ecosystems is thus likely attained through multiple modes of action. Furthermore, the effects of Pb on ecosystems necessarily begin with some initial effects at the molecular level of specific organisms within the ecosystem (U.S. EPA, 1986b).

Experimental settings for studies used in making causal determinations for the ecological effects of Pb include controlled exposures in the laboratory, microcosm experiments and field observations. Controlled exposure studies in laboratory or small-to medium-scale field settings provide the most direct evidence for causality, but their scope of inference may be limited. In contrast, microcosms and field studies where exposure is not controlled include potentially confounding factors (e.g., other metals) or factors known to interact with exposure (e.g., pH), thus increasing the uncertainty in associating effects with exposure to Pb specifically. A large majority of the available studies of Pb exposures are laboratory toxicity tests on single species, in which an organism is exposed to a known concentration of Pb and the effect on a specific endpoint is evaluated. These studies provide evidence for a temporal sequence between Pb exposure and an effect, an aspect important in judging causality (Table I of the Preamble). As detailed in the Framework for Causality (see Preamble), coherence between different types of studies also provides strong support to a determination of causality. Evidence from laboratory studies conducted under controlled conditions provides the largest amount of information used in the causal determinations summarized in Table 6-3, but their coherence with microcosm and field-based studies plays an important role in those determinations. Biological gradients (Table I of the Preamble) are often found in studies of the effects of Pb, and add support to causality where present. For some ecological endpoints, support for causal determinations is additionally supported by toxicological findings reviewed in the chapters of the ISA that evaluate evidence for human health effects associated with Pb exposure, particularly when a common mode of action is documented.

The amount of Pb in ecosystems is a result of a number of inputs and it is not currently possible to determine the contribution of atmospherically-derived Pb from total Pb in terrestrial, freshwater or saltwater systems. The causal determinations are, therefore, not specific to Pb from atmospheric deposition since atmospherically-derived Pb may ultimately be present in water, sediments, soils and biota (Section 6.2 and Figure 6-1). The causal determinations encompass findings of studies at concentrations of Pb reported from environmental media (Table 6-2), and up to one to two orders of magnitude above the range of these values (Table II of the Preamble). Studies at the upper range of Pb concentrations are generally conducted at and near heavily exposed sites such as mining and metal industries-disturbed areas. Studies at those higher concentrations were used

only when they were part of a range of concentrations that also included more typical values, or when they informed understanding of modes of action and illustrated the wide range of sensitivity to Pb across taxa.

The exposure values at which Pb elicits a specific effect in terrestrial and aquatic systems are difficult to establish, due the influence of other environmental variables on Pb bioavailability and toxicity and to substantial differences among biological species in their sensitivity to Pb. In the 1977 Pb AQCD (U.S. EPA, 1977), no correlation could be established between toxic effects in invertebrates, fish, birds or small mammals and environmental concentrations of Pb. At the time of the 1986 Pb AQCD additional data were available on toxicity but there was still little information on the exposure values that can cause toxic effects in small mammals or birds (U.S. EPA, 1986b). In the 2006 Pb AQCD (U.S. EPA, 2006c) several studies on effects of Pb exposure on natural ecosystem structure and function advanced the characterization of Pb levels in the environment that occur near contaminated sites (i.e., smelters, mining, industry). According to the 2006 Pb AQCD, natural terrestrial ecosystems near significant Pb sources exhibited a number of ecosystem-level effects, including decreased species diversity, changes in floral and faunal community composition, and decreasing vigor of terrestrial vegetation. These findings were summarized in Table AX7-2.5.2 of the Annex to the 2006 Pb AQCD (U.S. EPA, 2006c). The 2006 Pb AQCD concluded that, in general, there was insufficient information available for single materials in controlled studies to permit evaluation on higher levels of biological organization (beyond the organism). Furthermore, Pb rarely occurs as a sole contaminant in natural systems making the effects of Pb difficult to ascertain. Recent information available since the 2006 Pb AQCD, includes additional field studies in both terrestrial and aquatic ecosystems, but the connection between air concentration and ecosystem exposure continues to be poorly characterized for Pb and the contribution of atmospheric Pb to specific sites is not clear.

Table 6-3 Summary of Pb causal determinations for plants, invertebrates and vertebrates.

Level		Effect	Terrestrial[a]	Freshwater[a]	Saltwater[a]
Community and Ecosystem		Community and Ecosystem Effects	Likely Causal	Likely Causal	Inadequate
Population–Level Endpoints	**Organism–Level Responses**	Reproductive and Developmental Effects-Plants	Inadequate	Inadequate	Inadequate
		Reproductive and Developmental Effects-Invertebrates	Causal	Causal	Suggestive
		Reproductive and Developmental Effects-Vertebrates	Causal	Causal	Inadequate
		Growth-Plants	Causal	Likely Causal	Inadequate
		Growth-Invertebrates	Likely Causal	Causal	Inadequate
		Growth-Vertebrates	Inadequate	Inadequate	Inadequate
		Survival-Plants	Inadequate	Inadequate	Inadequate
		Survival- Invertebrates	Causal	Causal	Inadequate
		Survival- Vertebrates	Likely Causal	Causal	Inadequate
		Neurobehavioral Effects-Invertebrates	Likely Causal	Likely Causal	Inadequate
		Neurobehavioral Effects- Vertebrates	Likely Causal	Likely Causal	Inadequate
	Sub-organismal Responses	Hematological Effects-Invertebrates	Inadequate	Likely Causal	Suggestive
		Hematological Effects-Vertebrates	Causal	Causal	Inadequate
		Physiological Stress-Plants	Causal	Likely Causal	Inadequate
		Physiological Stress-Invertebrates	Likely Causal	Likely Causal	Suggestive
		Physiological Stress-Vertebrates	Likely Causal	Likely Causal	Inadequate

[a]Conclusions are based on the weight of evidence for causal determination in Table II of the ISA Preamble. Ecological effects observed at or near ambient Pb concentrations measured in soil, sediment and water in the most recent available studies (Table 6-2). were emphasized and studies generally within one to two orders of magnitude above the reported range of these values were considered in the body of evidence for terrestrial (Section 6.3.12), freshwater (Section 6.4.12) and saltwater (Section 6.4.21) ecosystems.

6.6 Supplemental Material

Table 6-4 Recent evidence for Pb effects on terrestrial plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Exposure Concentration (Nominal)	Exposure Concentration (Measured)	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Plants							
Buckwheat (*Fagopyrum esculentum*)		**Contaminated soil**: HCl extractable: 6,643 mg Pb/kg Acetate extractable: 832 mg Pb/kg Water leachate: 0.679 mg Pb/kg **Control soil:** HCl extractable: 5 mg Pb/kg Acetate extractable: ND Water leachate: ND	Plants were grown for 8 weeks in contaminated soil collected from a shooting range, and control soil.	**Contaminated soil** Sand: 62.3% Silt: 36.7% Clay: 1.0% pH: 6.0 CEC: 13.0 **Control soil** Sand: 87.7% Silt: 12.3% Clay: ND pH: 6.3 CEC: 7.6	**Growth:** No effect on growth **Survival:** No effect on survival		Tamura et al. (2005)

Table 6-4 (Continued): Recent evidence for Pb effects on terrestrial plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Exposure Concentration (Nominal)	Exposure Concentration (Measured)	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Canola (*Brassica napus*)	0; 22; 45; and 67 mg Pb/kg		Plants of four cultivars were grown for 40 days in soil amended with Pb chloride.		**Growth:** Shoot and root dry weight decreased with increasing Pb Zn, Cu, Fe, Mn content decreased with increasing Pb. N, P, K, and Ca^{2+} content decreased to a lesser degree.		Ashraf et al. (2011)
Chinese cabbage (*Brassica pekinensis*)		46; 874; 1,703 mg Pb/kg dry soil	Plants were grown for 12 days in soil amended with Pb acetate.		**Growth:** Shoot biomass decreased with increasing Pb (91% and 84% of lowest exposure).		Xiong et al. (2006)
Corn (*Zea mays*)	0; 0.007; 0.7; 7 mg Pb/L		Seeds were germinated on paper soaked in P-sulfate. Plants were grown for 21 days in washed sand with Pb-sulfate amended nutrient solution.		**Reproduction:** Germination%, germination index, plant decreased with increasing Pb. **Growth:** Shoot length, plant dry weight, water use efficiency decreased with increasing Pb.		Ahmad et al. (2011)

Table 6-4 (Continued): Recent evidence for Pb effects on terrestrial plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Exposure Concentration (Nominal)	Exposure Concentration (Measured)	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Grass pea (*Lathyrus sativus*)	16; 31; 63; 125; 188 mg Pb/L		Plants were grown in soil amended with Pb nitrate.		**Reproduction:** Germination decreased with increasing Pb (control 100%, highest exposure 30%). Chromosomal abnormalities increased with increasing Pb (control 0%, highest exposure 72%). **Growth:** Shoot length decreased with increasing Pb (highest exposure was 50% of control).		Kumar and Tripathi (2008)
Lettuce (*Lactuca sativa*)		2,000 mg Pb/kg, but soil was mixed with 50% V/V vermiculite following amendment with Pb nitrate. Tissue Pb: 3.22-233 mg Pb/kg	Plants were grown for 40 days in 21 soils with varying native CEC, OC, pH, and amorphous Fe and Al oxides, which were then all amended with Pb nitrate and mixed with 50% V/V vermiculite.	After amendment: pH: 3.8-7.8 CEC: 3.01-32.04 $cmol_c$/kg OC: 5 - 30 g/kg Fe/Al oxides: 0.009-0.195 mol/kg	**Reproduction:** Germination 50 – 92% Germination was greater in amended soils. **Growth:** 2.5 – 88.5% of control In the presence of the same amount of $Pb(NO_3)_2$, OC was the main determinant of effects, although CEC had a strong influence, but mediated by its effect on pH and Fe/Al oxides		Dayton et al. (2006)

Table 6-4 (Continued): Recent evidence for Pb effects on terrestrial plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Exposure Concentration (Nominal)	Exposure Concentration (Measured)	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Mustard (*Brassica juncea*)	0;31; 62; 124; 186; 249; 311 mg Pb/L		Plants were grown for 60 days in field soil amended with Pb acetate.		**Growth:** Root and shoot length decreased with increasing Pb, and the decrease was greater with time. After 60 days, roots were two times longer in controls than in the highest Pb exposure shoot length was 75% greater.		John et al. (2009)
Radish (*Raphanus sativus*)	0; 21; 105 mg Pb/L		Plants were grown for 35 days in sand with a full nutrient solution amended with Pb nitrate.		**Growth:** Leaf area, root volume, shoot and root dry weight decreased with increasing Pb. (total dry weight at 21 mg Pb/L was 30% smaller than control, 52% smaller at 105 mg Pb/L).		Gopal and Rizvi (2008)
Wheat (*Triticum aestivum*) Lettuce (*Lactuca sativa*)		69-9,714 mg Pb/kg	Wheat plants were grown for 6 weeks in undisturbed core samples from four locations in each two Pb-contaminated sites Lettuce seeds were germinated in the leachate.	pH: 4.25-7.26 OC: 6.2-47.6%	**Growth:** No effects were found on germination or growth of either species.		Chapman et al. (2010)

Table 6-4 (Continued): Recent evidence for Pb effects on terrestrial plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Exposure Concentration (Nominal)	Exposure Concentration (Measured)	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Invertebrates							
Cabbage aphid (*Brevicoryne brassicae*)	0.87 mg Pb/L in watering solution used for plants		Aphids were reared for several generations on radish and cabbage plants grown in soil amended with Pb nitrate.		**Reproduction:** In aphids fed Pb-contaminated plants, development time was longer, and relative fecundity and rate of population increase were lower than in control aphids. **Survival:** Mortality was higher in exposed aphids, both adults and offspring.		Görür (2007)

Table 6-4 (Continued): Recent evidence for Pb effects on terrestrial plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Exposure Concentration (Nominal)	Exposure Concentration (Measured)	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Collembolan (*Folsomia candida*)		Approximate range was 12 mg Pb/kg soil to 15,000 mg Pb/kg soil; Pore water approximately 0.002 Pb/L to 1,000 mg Pb/L (Concentrations were measured, but not reported).	Springtails were reared for 28 days in soil collected at seven locations along each of three transects with increasing Pb concentrations within each transect (21 locations). Lowest concentration soils from each of the three transects were then amended with Pb nitrate to match the gradient, and one set of the amended samples were then leached, for a total of 57 concentrations of Pb.	pH was constant in transects, but decreased with increasing addition of $Pb(NO_3)_2$ in both amended and amended-and-leached soils. pH decreased by 3 units in the highest addition, regardless of subsequent leaching.	**Reproduction:** Reproduction decreased by up to 50% in transect soils Amended soils Pb concentrations 2,207 mg Pb/kg or lower never had a significant effect on reproduction.	Transect **A 28 day** EC_{50} in mg Pb/kg dry weight: native: >5,690 amended: 2,570 amended and leached: 2,060 Transect **B 28 day** EC_{50} in mg Pb/kg dry weight: native: >14,400 amended: 3,210 amended and leached: 2,580 Transect **C 28 day** EC_{50} in mg Pb/kg dry weight: native: >5,460 amended: 2,160 amended and leached: 2,320	Lock et al. (2006)
Collembolan (*Folsomia candida*)	0; 100; 200; 400; 800; 1,600; 3,200 mg Pb/kg dry soil		Springtails were reared for 10 days in field soil amended with Pb chloride.		**Reproduction:** Hatching success decreased with increasing Pb.	EC_{50} (hatching): 2,361 mg Pb/kg dry soil	Xu et al. (2009b)

Table 6-4 (Continued): Recent evidence for Pb effects on terrestrial plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Exposure Concentration (Nominal)	Exposure Concentration (Measured)	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Collembolan (*Sinella curviseta*)	0; 100; 200; 400; 800; 1,600; 3,200 mg Pb/kg dry soil		Springtails were reared for 28 days in field soil amended with Pb chloride.		There was a small but not significatnt effect of Pb on growth, and no effect on survival and reproduction.	**Survival**: LC_{10}/EC_{10}: 1,838 mg Pb/kg **Reproduction**: LC_{10}/EC_{10}: 642 mg Pb/kg EC_{50}: 3,212 mg/kg Pb **Body Size**: LC_{10}/EC_{10}: 4,094 mg Pb/kg	Xu et al. (2009a)
Collembolan (*Paronychiurus kimi*)	Toxicity run: 100; 500; 1,000; 2,000 mg Pb/kg Reproduction run: 0; 250; 500; 1,000; 2,000; 3,000 mg/kg Pb		Springtails were reared for 28 days on artificial soil amended with Pb chloride in two separate runs.		**Survival:** Survival decreased with increasing Pb. **Reproduction:** Offspring production and instantaneous rate of increase values decreased with increasing Pb.	**Survival LC_{50}:** 7 day: 1,322 mg Pb/kg 28 day: 1,299 mg Pb/kg **EC_{50}** 28 day: 428 mg Pb /kg NOEC r**eproduction**: EC_{50} 28 day: 428 mg Pb/kg NOEC: 250 mg Pb/kg LOEC: 500 mg Pb/kg	Son et al. (2007)

Table 6-4 (Continued): Recent evidence for Pb effects on terrestrial plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Exposure Concentration (Nominal)	Exposure Concentration (Measured)	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Collembolans (*Sinella coeca, Folsomia candida*)	10; 50; 100; 500; 1,000 mg Pb/kg		Springtails were reared for 42 or 45 days in artificial soil amended with Pb nitrate.		***S. coeca*:** **Survival:** Mortality significantly increased with increasing concentration in adult population. **Reproduction:** Juvenile production not significantly compromised at 10-500 mg Pb/kg, reduced at 1,000 mg Pb/kg ***F. candida*:** **Survival:** Increase in mortality with increasing concentration **Reproduction:** Juvenile production not significantly reduced between 10-500 mg Pb/kg, significant effect at 1,000 mg Pb/kg	**LC_{50}:** ***S. coeca*:** Could not be determined ***F. candida*:** Could not be determined **EC_{50} reproduction**: ***S. coeca*:** 490 mg Pb/kg Pb on dry soil ***F. candida*:** Could not be calculated with accuracy; Ranged from 500-1,000 mg Pb/kg	Menta et al. (2006)

Table 6-4 (Continued): Recent evidence for Pb effects on terrestrial plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Exposure Concentration (Nominal)	Exposure Concentration (Measured)	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Earthworm (*Eisenia andrei*)		2,000 mg Pb/kg in soil Internal concentration of Pb in earthworms varied among the amended soils, between 29 and 782 mg Pb/kg dry weight.	Earthworms were reared for 28 days in 21 soils with varying native CEC,OC, pH, and amorphous Fe and Al oxides, which were then all amended with Pb nitrate.	After amendment pH: 3.8 - 7.8 CEC: 3.01 - 32.04 $cmol_c$/kg OC: 5 - 30 g/kg Fe/Al oxides: 0.009 - 0.195 mol/kg	**Survival:** Mortality ranged between 0 and 100%. In the presence of potentially lethal amounts of Pb, the main determinant of mortality was pH, with little or no effect from OC, CEC, or Fe/Al oxides. **Reproduction:** Reproduction relative to controls ranged between 0 and 167%. Effects of Pb on reproduction are dependent principally on Fe/Al oxides, with some influence of CEC		Bradham et al. (2006)
Earthworm (*Eisenia fetida*)		18-9,311 mg Pb/kg	Earthworms were reared for 14 days in OECD-standard toxicity testing soil with 7 concentrations of Pb, either one or 10 earthworms per container.	pH decreased with increasing Pb nitrate addition NH_3 concentration increased with Pb concentration and time	**Survival:** Mortality increased from 0 to 100% with increasing Pb, with 100% reached at 4,500 mg Pb/kg after 7 days and 14 days. Number of worms per container had no effect on mortality. **Growth:** Worm weight decreased with increasing Pb, and faster in multiple-worm containers.	LC_{50} (multiple-occupancy): 2,662 mg Pb/kg at 7 days and 2,589 mg Pb/kg at 14 days or 2,827 mg Pb/kg at both 7 and 14 days	Currie et al. (2005)

Table 6-4 (Continued): Recent evidence for Pb effects on terrestrial plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Exposure Concentration (Nominal)	Exposure Concentration (Measured)	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Earthworm (*Eisenia fetida*)	0; 300; 711; 1,687; 2,249 mg Pb/kg	Mean: 79% of nominal	Earthworms were reared for 14 days in soil amended with five levels of Pb nitrate and artificially aged.	pH 6.72 prior to amendment OC 0.7% CEC 11 meq/100 g	**Survival:** Mortality was only observed at the highest exposure.		Jones et al. (2009b)
Earthworm (*Eisenia fetida*)	Soil 1: 0; 355; 593; 989; 1,650 mg Pb/kg Soil 2: 59; 297; 593; 2,965 mg Pb/kg Soil 3: 386; 771; 1,929; 3,857 mg Pb/kg		Earthworms were reared for 28 days in three soils amended with five levels of Pb nitrate without aging, after which they were removed from the containers. Containers were then kept in the same conditions for another 28 days, after which cocoons were extracted.	pH 6.72; 5.48; 6.75 (prior to amendment) OC 0.7; 1.2; 5.2% CEC 11; 8; 27 meq/100g	**Reproduction:** Soil 1: Juvenile and cocoon count decreased from 19 and 45, respectively, to near 0 with increasing Pb. Soil 2: Cocoon count decreased to 40% of control at highest Pb. Soil 3: Cocoon count was 0 at all concentrations.		Jones et al. (2009b)

Table 6-4 (Continued): Recent evidence for Pb effects on terrestrial plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Exposure Concentration (Nominal)	Exposure Concentration (Measured)	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Earthworm (*Pheretima guillelmi*)	Toxicity run: 0, 1,000; 1,400; 2,000; 2,800; 3,800; 5,400; 7,500 mg Pb/kg dry weight Sublethal toxicity run: 1,000; 1,400; 1,800; 2,500 mg Pb/kg dry weight		Earthworms were reared for 14 days in OECD-standard soil amended with Pb nitrate. There were two runs with different concentrations.		**Survival:** Mortality increased with increasing Pb.(0% in control, 100% at 7,500 mg Pb/kg after 14 days). **Growth:** Weight decreased with increasing Pb (2.5 g in control, 1.4 g at 5.400 mg Pb/kg after 14 days). **Reproduction:** Sperm abnormalities increased with increasing Pb (10% of control, 21% at 2,500 mg Pb/kg after 14 days).	**LC_{50}** : 4,285 mg Pb/kg at 7 days and 3,207 mg Pb/kg at 14 days	Zheng and Li (2009)
Earthworms (*Eisenia andrei, Lumbricus rubellus, Aporrectodea caliginosa*)		0; 1,000; 3,000; 4,000; 5,000; 7,500; 10,000 mg Pb/kg	Earthworms were reared for 28 days in sterilized Kettering Loam watered with Pb nitrate solution.	Temperature: 20 °C (*E. andrei*); 15 °C (*L. rubellus* and *A. caliginosa*) pH Day 7 : 4.57-5.83, Day 28: 4.71-5.83, increasing with decreasing Pb	**Growth:** Weight decreased with increasing concentration and time, severity of weight decrease varied with species. **Survival:** Mortality increased with increasing concentration and time, and varied with species 100% mortality for all species at higher concentrations after 28 days.	**LC_{50}:** ***E. andrei*:** 5,824 mg Pb/kg ***L. rubellus*:** 2,867 mg Pb/kg ***A. caliginosa*:** 2,747mg Pb/kg **EC_{50}:** ***E. andrei*:** 2,841 mg Pb/kg ***L. rubellus:*** 1,303 mg Pb/kg ***A. caliginosa*:** 1,208 mg Pb/kg	Langdon et al. (2005)

Table 6-4 (Continued): Recent evidence for Pb effects on terrestrial plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Exposure Concentration (Nominal)	Exposure Concentration (Measured)	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Nematode (*Caenorhabditis elegans*)		0.5; 10; 21 mg Pb/L	Nematodes at various developmental stages were exposed to $Pb(NO_3)_2$ for four hours in water. Late larval nematodes (L4) were exposed for one or three days.		**Reproduction:** Brood size decreased with increasing Pb, but the decrease was smaller with increasing developmental age. Generation time increased with increasing Pb, and the increase was smaller with increasing developmental age. These effects were greater in late larval nematodes when exposure duration increased from four hours to one and three days.		Guo et al. (2009)
Nematode (*Caenorhabditis elegans*)	5; 10; 16; 21 mg Pb/L		Nematodes were placed for 48 hours in growing medium with 4 concentrations of Pb.		**Survival:** No effect		Vigneshkumar et al. (2013)
Nematode (*Caenorhabditis elegans*)	0.5; 16; 41 mg Pb/L		Nematodes were placed for three days in growth medium amended with Pb nitrate.		**Growth:** Life span, body size decreased with increasing Pb. **Reproduction:** Generation time and brood size increased with increasing Pb. All effects were present and of comparable magnitude in progeny of exposed nematodes.		Wang and Yang (2007)

Table 6-4 (Continued): Recent evidence for Pb effects on terrestrial plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Exposure Concentration (Nominal)	Exposure Concentration (Measured)	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Snail (*Achatina achatina*)		1.33; 70.98; 134.61; 339.40; 674. 86; 1,009.22; 1,344.39 mg Pb/kg	Snails were reared for 12 weeks on a diet amended with Pb chloride.		**Survival:** no effect **Growth:** Small decrease in feeding at highest exposure, small decrease in weight gain with increasing Pb (over 12 weeks, snails in the highest exposure gained 12% less weight than in the lowest exposure).		Ebenso and Ologhobo (2009a)
Snail (*Achatina achatina*)		0.56; 20.37; 200.42; 1,200.30 mg Pb/kg	Snails from laboratory source were reared for 12 weeks in bottomless enclosures at four locations within the grounds of an abandoned battery factory.	pH: 4.42 - 6.29, decreasing with increasing Pb OC: 1.39 - 3.45%, decreasing with increasing Pb CEC: 3.32 - 5.37 cmol/kg, increasing with increasing Pb.	**Growth:** Feeding, weight gain and shell thickness all decreased with increasing Pb (13; 17; and 19% lower in highest exposure than in lowest).		Ebenso and Ologhobo (2009b)
Snail (*Theba pisana*)	0; 50; 100; 500; 1,000; 5,000; 10,000; 15,000 mg Pb/kg		Snails were reared for 5 weeks on Pb-amended diet.		**Growth:** Feeding and weight gain decreased with increasing Pb and time (snails in 0 added Pb gained 45% more weight than in highest Pb). **Survival:** No effect		El-Gendy et al. (2011)

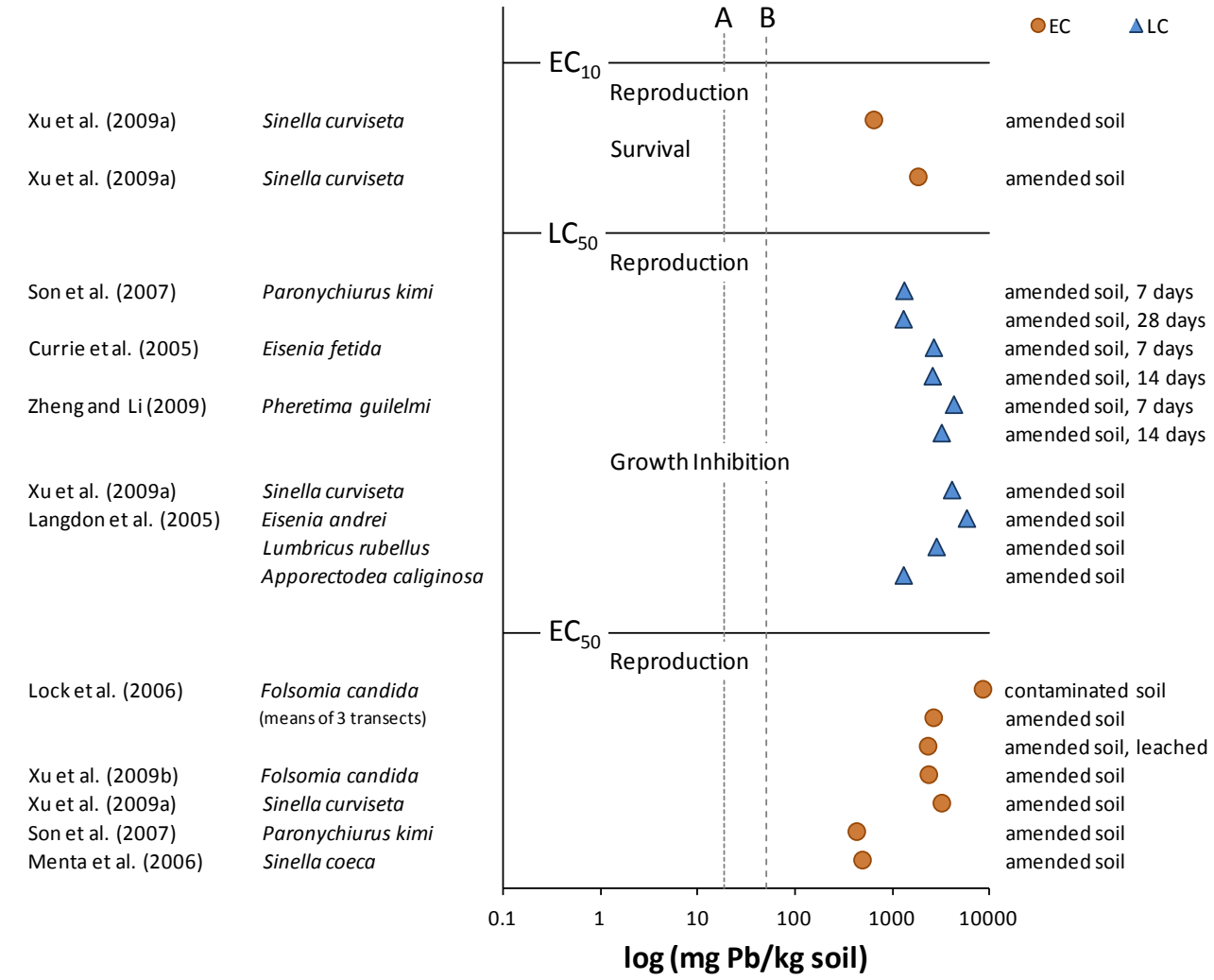
Table 6-4 (Continued): Recent evidence for Pb effects on terrestrial plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Exposure Concentration (Nominal)	Exposure Concentration (Measured)	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Snails (*Cantareus aspersus, Helix aspersa*)		Total Soil Pb: 1740-2060 mg Pb/kg $CaCl_2$ extractable; 4-80 mg Pb/kg Dissolved (estimated): 0.007-0.09 mg Pb/L	Snails were reared for 7 - 9 weeks in field soil amended with varying amounts of Pb-sulfate, clay, peat, and $CaCO_3$.	Clay content 11 - 16% Organic matter 1.2 - 10% pH 4.6 - 7.49	**Growth:** No effect **Survival:** No effect		Pauget et al. (2011)
Vertebrates							
Japanese quail (*Coturnix coturnix japonica*)		Drinking water Pb: 0; 5; 50 mg Pb/L Tissue Pb: 0;1.1; 10.7 mg/kg wet weight	Quail were given Pb-amended water for 7 weeks.		**Growth:** Feed intake and growth rate not affected. **Survival:** Morbidity/mortality was lower in highest exposure than in control. Incidence of pericarditis, airsacculitis, perihepatitis, and arthritis was lower in highest exposure than in control.		Nain and Smits (2011)

Table 6-4 (Continued): Recent evidence for Pb effects on terrestrial plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Exposure Concentration (Nominal)	Exposure Concentration (Measured)	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Pied flycatchers (*Ficedula hypoleuca*)		**Blood Pb in nestlings at mining site** while active: 41 mg Pb/100 kg wet weight; after closing: 29 mg Pb/100 kg wet weight. **Blood Pb in nestlings at reference site** while active: 2 mg Pb/100 kg wet weight; after closing: 0.4 mg Pb/100 kg wet weight.	Data were collected in wild flycatchers near a Pb mine and at a reference site for three years while the mine was active, and for three years five years after mine closing.		**Reproduction:** Clutch size and breeding success were lower at the mine site, but did not change after closure of the mine (clutch size 5.6 reference, 4.9 mining site; breeding success 80% reference site, 76% mining site). Nestling mortality was higher at the mine site, and increased after closure (5% reference site, 11% mining site while active; 11% reference site, 26% mining site after closure).		Berglund et al. (2010)
Pig (*Sus domestica*)		**Feed Pb** control: unreported exposed: 10 mg Pb/kg **Blood Pb** control: 1.44 µg/dL exposed: 2.08 µg/dL	Pigs were reared for 120 days with Pb-sulfate-amended feed.		**Growth:** Significant decrease in body weight, average day gain, average day feed intake, and feed efficiency. Increase in feed conversion ratio. **Reproduction:** No effect on ovary and uterus weight		Yu et al. (2005)

[a]References included are those which were published since the 2006 Pb AQCD.



Note: Detailed summaries of the individual studies referenced above, including experimental conditions, modifying factors and responses at higher concentrations are summarized in Table 6-4. Data in Figure 6-4 are limited to EC and LC values published since the 2006 Pb AQCD. Reference concentrations "A" and "B" are the National mean background soil Pb sampled between 1961 and 1997, as reported in U.S. EPA (2007d, 2006b, 2003b), and the 95th percentile of background soil Pb sampled between 1961 and 1976 in the contiguous U.S. (Shacklette and Boerngen, 1984) (see Table 6-2).

Figure 6-4 Subset of concentration-response data reported in Table 6-4 for Pb effects on growth, reproduction, and survival in some terrestrial invertebrates.

Table 6-5 Recent evidence for Pb effects on freshwater plants, invertebrates and vertebrates; growth, reproduction, and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Algae/Plants						
Blue-green algae *(Spirulina (Arthrospira) platensis)*	5,000; 10,000; 30,000; 50,000; and 100,000 µg Pb/L (nominal concentration at day 0 and then Pb in media was measured every two days thereafter).	10-day exposure to Pb nitrate in Zarrouk liquid medium.	Temperature: 25 ± 1 °C pH: 7.0 Light dark cycle of 14:10 hours.	**Growth:** 10-day algal growth (measured turbidimetrically at 560 nm) was stimulated by 3.7% in the lowest concentration, growth was inhibited at higher concentrations of 30,000; 50,000; and 100,000 µg Pb/L by 40; 49; and 78%, respectively. Chlorophyll *a* and *b* content were significantly diminished at the three highest exposures.	**LC_{50}**: 75,340 µg/L	Arunakumara et al. (2008)
Microalgae *(Scenedesmus obliquus)*	5,000 to 300,000 µg Pb/L (nominal)	48 and 96-hour acute toxicity test with Pb nitrate in BG11 medium.	Plants were incubated at normal room temperature	**Growth:** In growth studies (measured as cell division rate) *S. obliquus* was significantly more sensitive to Pb exposure than *C. vulgaris*	**48 hour EC_{50}:** 4,040 µg Pb/L *S. obliquus*	Atici et al. (2008)
Microalgae *(Chlorella vulgaris)*			(not provided)		**48 hour EC_{50}:** 24,500 µg/L *C. vulgaris*	

Table 6-5 (Continued): Recent evidence for Pb effects on freshwater plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Duckweed *(Lemna minor)*	100; 200; 490; 900; 2,000; 5,020; 7,990; and 9,970 µg Pb/L (measured)	4 day or 7-day exposures to Pb chloride in static test conditions with Jacob culture medium under continuous illumination.	Temperature: 25 ± 2 °C pH 6.0	**Growth:** Growth (measured as biomass) of the duckweed was promoted up to 103% at 100 µg Pb/L and 200 µg Pb/L. However, growth was inhibited monotonically at all other test levels with increasing concentrations. Overall, the relative growth rate was reduced to 37–38% at the highest concentration.	**4 day EC_{50}:** 6,800 µg Pb/L **7 day EC_{50}:** 5,500 µg/L	Dirilgen (2011)
Duckweed *(Lemna minor)*	2,070; 10,360; 20,700; and 103,600 µg Pb/ L (nominal)	9-day exposures to Pb nitrate in a growth chamber on Knopp's medium under a 14-hour photoperiod.		**Growth:** At lower Pb doses, growth was slightly stimulated. Fresh weight was lower by 65% at the highest dose. Pb-induced chlorosis occurred and the enzymes of the antioxidative system were modified due to Pb exposure in all concentrations.		Paczkowska et al. (2007)
Duckweed *(Wolffia arrhiza)*	210; 2,120; 20,720; and 207,200 µg Pb/ L (analytically verified)	14-day exposure to Pb as Pb nitrate in sterile 1/50 dilution of Hutner's medium, day to night cycle of 16:8 hours.	Temperature: 25 ± 0.5 °C pH: 7.0	**Growth:** Biomass decreased proportionally with increasing Pb concentration; Chlorophyll *a* content was significantly inhibited at 210 µg Pb/L and greater; carotenoid, monosaccharide and protein content significantly decreased in higher concentrations.		Piotrowska et al. (2010)
Waterweed *(Elodea canadensis)*	1,000; 10,000; and 100,000 µg/L (nominal)	Plants were exposed 5 days to Pb as Pb acetate in a 10% nutrient solution and then assayed for pigment content, total ascorbic acid, and protein content.	Temperature: 25–27°C pH 6.5-6.7 10% Hoagland & Arnon nutrient solution	**Growth:** The chlorophyll, carotenoid, and protein contents of *E. canadensis* were significantly reduced following Pb accumulation.		Dogan et al. (2009)

Table 6-5 (Continued): Recent evidence for Pb effects on freshwater plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Wetland plants *(Beckmannia syzigachne, Alternanthera philoxeroides, Juncus effusus, Oenanthe javanica, Cyperus flabelliformis, Cyperus malaccensis, Polypogon fugax, Leersia hexandra, Panicum paludosum, Neyraudia reynaudiana)*	20,000 µg/L (nominal) Metals were analyzed in plant samples.	Field-collected tillers or seedlings (from various locations in China) for each species were used in 21-day experiments to determine Pb tolerance as inferred from measuring the elongation of the longest root in a hydroponic system in a Pb nitrate solution.		**Growth:** Root elongation was significantly reduced in a number of wetland species (*B. syzigachne, J. effusus, O. javanica, C. flabelliformis, C. malaccensis, and N. reynaudiana*). Metal tolerance was related to root anatomy and spatial pattern of radical oxygen loss.		Deng et al. (2009)
Invertebrates						
Rotifer *(Brachionus calyciflorus)*	67; 194; 284; 390; and 700 µg Pb/L (measured)	Cysts of rotifers were obtained from Florida Aqua farms in Dade City, Florida, U.S. Tests with Pb nitrate were performed in total darkness for 48 hours.	Temperature: 25 ± 1 °C pH: 8.19	**Reproduction:** The total number of rotifers and the intrinsic rate of population increase exhibited concentration-dependent responses at the end of the 48 hour incubation period.	**EC_{20} for number of rotifers:** 125 µg Pb/L **48-hour EC_{20} for intrinsic rate of population increase:** 307 µg Pb/L **NOEC:** 194 µg Pb/L **LOEC:** 284 µg Pb/L	Grosell et al. (2006b)

Table 6-5 (Continued): Recent evidence for Pb effects on freshwater plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Rotifer *(Brachionus patulus)*	1,250; 2,500; 4,000; 5,000; and 8.000 µg Pb/L (nominal) for acute toxicity tests. Chronic exposures used nominal concentration of 60 and 600 µg Pb/L with varying turbidity levels.	24-hour exposures to Pb chloride in the presence and absence of sediments using rotifers originally isolated from the Chimaliapan wetland, Toluca, Mexico. Three week chronic toxicity tests were also conducted.	Temperature: 20 °C	**Reproduction:** In chronic tests, net reproductive rate and rate of population increase decreased under conditions of increasing turbidity and Pb concentration. **Survival:** 24-hour LC_{50} reported for this species. In chronic tests, average life span and life expectancy at birth decreased under conditions of increasing turbidity and Pb concentration.	**24-hour LC_{50}**: 6,150 µg/L	García-García et al. (2007)
Rotifer *(Euchlanis dilatata)*	0.1; 0.5; 50; 100; 250; 1,000; 2,500 µg Pb/L (analytically verified, actual concentrations not reported)	48-hour acute toxicity tests with rotifer neonates exposed to Pb nitrate in synthetic moderately hard water. Adult rotifers were collected in a reservoir in Aguascalientes, Mexico.	Temperature: 25 ± 2 °C pH: 7.5 Hardness: 80–100 mg/L $CaCO_3$	**Survival:** Based on 48-hour LC_{50} *E. dilatata* is among the most sensitive rotifer species to Pb. *E. dilatata* may be a more suitable test organism for ecotoxicology in Mexico, where this study was conducted, instead of *D. magna,* a species that is not been found in Mexico reservoirs.	**48-hour** **NOEC**: 0.1 µg/L **LOEC**: 0.5 µg/L **LC_{50}:** 35 µg/L (estimated from analytically verified concentrations)	Arias-Almeida and Rico-Martínez (2011)

Table 6-5 (Continued): Recent evidence for Pb effects on freshwater plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Cladoceran *(Ceriodaphnia dubia)*	Measured but not reported. Predicted concentration of major Pb chemical species in the natural water bioassays is provided in Table 4 of Esbaugh et al. (2011).	Acute toxicity of Pb to *C. dubia* was assessed in 48-hour exposures to two lab generated reference waters and eight natural waters from across North America selected to include a range of water quality parameters. Waters were spiked with varying concentrations of Pb as Pb nitrate.	Temperature: 26°C Water chemistry of the field-collected waters are reported in Table 1 of Esbaugh et al. (2011) including pH (range 5.5 to 8.5), Ca^{2+} (range 24 to 1,934 µM), DOC (range 36 to 1,244 µM) and hardness (range 4 to 298 mg/L)	**Survival:** LC_{50} values ranged from 29 to 1,180 µg Pb/L. Sensitivity to Pb varied greatly with water chemistry. DOC was correlated with protection from acute toxicity.	**48-hour LC_{50} range:** 29 to 1,180 µg Pb/L **NOEC range:** 18 to <985 µg Pb/L. **LOEC range:** 52 to 1,039 µg Pb/L	Esbaugh et al. (2011)
Cladoceran *(Ceriodaphnia dubia)*	A range of 5 to 6 Pb concentrations (measured but not reported) were prepared with varying pH, hardness and alkalinity.	Chronic 7-day static renewal 3 times per week in 2:1 dechlorinated, aerated tap water:deionized water to determine the effects of hardness (as $CaSO_4$ and $MgSO_4$), alkalinity, pH, and DOM on Pb toxicity.	Temperature: 25 °C pH: 6.4-8.2 Hardness: 22-524 mg/L	**Survival:** DOM and alkalinity have a protective effect against chronic toxicity of Pb. $CaSO_4$ and $MgSO_4$ do not have a protective influence of water hardness; Pb toxicity increased at elevated Ca^{2+} and Mg^{2+}. Low pH increases the toxicity of Pb. **Reproduction:** Increased DOC leads to an increase in mean EC_{50} for reproduction ranging from approximately 25 µg Pb/L to >500 µg Pb/L.	**Control base water EC_{20}:** 45 µg Pb/L **5.0 mM $CaSO_4$ EC_{20}:** 22 µg Pb/L **32 mg/L DOM EC_{20}:** 523 µg Pb/L **2.5 mM $NaHCO_3$ EC_{20}:** 73 µg Pb/L Additional EC_{20} and all EC_{50} values were reported in the study.	Mager et al. (2011a)
Cladoceran *(Diaphanosoma birgei)*	2,000 to 5,500 µg Pb/L (analytically verified)	24-hour exposure to Pb chloride in moderately hard water.	Temperature: 23 °C pH: 7.0–7.5	**Survival:** LC_{50} reported	**24 hour LC_{50}** 3,160 µg Pb/L	García-García et al. (2006)

Table 6-5 (Continued): Recent evidence for Pb effects on freshwater plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Cladoceran *(Moina micrura)*	1,000 to 8,000 µg Pb/L (analytically verified)	24-hour exposure to Pb chloride in moderately hard water	Temperature: 23 °C pH: 7.0–7.5	**Survival:** *M. micrura* was more sensitive to Pb than *D. birgei* and *A. rectangular.*	**24 hour LC_{50}** 690 µg Pb/L	García-García et al. (2006)
Cladoceran *(Alona rectangular)*	3,000 to 10,000 µg Pb/L (analytically verified)	24-hour exposure to Pb chloride in moderately hard water	Temperature: 23 °C pH: 7.0–7.5	**Survival:** *A. rectangular* was more resistant to Pb than the other species tested	**24 hour LC_{50}** 7,000 µg Pb/L	García-García et al. (2006)
Cladoceran *(Daphnia magna)*	**Acute test:** Concentrations not provided **Chronic test:** 25 µg Pb/L 250 µg Pb/L 2,500 µg Pb/L (nominal)	24 hour acute toxicity test and 21 day toxicity test with Pb nitrate, static renewal every two days.	Temperature: 20 ± 1 °C	**Reproduction:** Significant concentration-dependent decrease in number of neonates per female; Significant long-term effects on reproduction. Negative correlation between hemoglobin gene expression and reproduction outcomes.	**24-hour EC_{50}** (immobility): 18,153 µg Pb/L	Ha and Choi (2009)
Cladoceran *(Daphnia pulex)*	**Acute test:** 250; 500; 1,000; 2,000; 5,000 µg Pb/L (nominal) **Chronic test:** 250; 500; 1,000 µg Pb/L (nominal).	48-hour acute toxicity test and two 21-day exposures to Pb nitrate in dechlorinated tap water.		**Reproduction:** Reproduction rates (cumulative neonates) significantly decreased at 1,000 µg Pb/L in the first chronic toxicity test and at 500 µg Pb/L in a second test. **Survival:** LC_{50} values reported	**48-hour LC_{50}:** 4,000 µg Pb/L	Theegala et al. (2007)

Table 6-5 (Continued): Recent evidence for Pb effects on freshwater plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Ostracod *(Stenocypris major)*	475; 1,160; 3,410; 4, 829; 8,972 µg Pb/L (measured)	96-hour static renewal with Pb nitrate in dechlorinated tap water. Ostracods were collected from a filter system of a fish pond in Bangi, Selangor, Malaysia.	Temperature: 28-30 °C pH: 6.5 ± 0.01 Conductivity: 244.3 ± 0.6 µS/cm DO: 6.3 ± 0.06 mg/L Total hardness: 15.6 mg/L as $CaCO_3$ Light dark cycle of 12:12 hours.	**Survival:** LC_{50} values reported	**24-hour LC_{50}:** 6,583 **µg** Pb/L **48-hour LC_{50}:** 2,886 µg Pb/L **72-hour LC_{50}:** 1,491 µg Pb/L **96-hour LC_{50}:** 526 µg Pb/L	Shuhaimi-Othman et al. (2011b)
Midge *(Chironomus tentans)*	42;109; 497; 724 µg Pb/L (measured)	27 day exposure with 8-day old larvae to Pb-nitrate in a mixture of tap water and deionized water supplemented with Ca^{2+} (as $CaSO_4$)	Temperature: 23 ± 1 °C pH: 7.9	**Survival:** Significantly reduced at the two highest concentrations	**NOEC:** 109 µg Pb/L **LOEC:** 497 µg Pb/L	Grosell et al. (2006b)

Table 6-5 (Continued): Recent evidence for Pb effects on freshwater plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Midge *(Chironomus dilutus,* formerly *C. tentans)*	29; 57; 75; 115; 128; 152 µg Pb/L (measured)	96-hour static renewal test 20 day midge life cycle test in Pb chloride spiked water, flow through, and emergence at 55 days. The *C. dilutus* culture was initially started with egg cases from Aquatic Biosystems, Fort Collins, CO, USA.	**Average ± SD (range):** Temperature: 22.2 ± 1.0 °C (19.7-24.4 °C) pH: 7.26 ± 0.21 (6.9 - 7.7) Hardness: 32 ± 3.2 mg/L as $CaCO_3$ Alkalinity: 31 ± 3.0 mg/L as $CaCO_3$ Conductivity: 76 ± 4.9 µs DO: 7.8 ± 0.8 mg/L	**Growth:** Growth and emergence decreased as concentration increased. **Reproduction:** No effect **Survival:** No effect	**96-hour LC_{50}:** 3,323 µg Pb/L **Survival** **NOEC:** 152 µg Pb/L **LOEC:** >152 µg Pb/L **MATC:** >152 µg Pb/L **Weight** **NOEC**: 57 µg Pb/L **LOEC:** 75 µg Pb/L **MATC:** 65 µg Pb/L **EC_{10}**: 15 µg Pb/L **EC_{20}**: 28 µg Pb/L **Fecundity** **NOEC:** 152 µg Pb/L **LOEC:** >152 µg Pb/L **MATC:** >152 µg Pb/L **EC_{10}**: >152 µg Pb/L **EC_{20}**: >152 µg Pb/L **Emergence** **NOEC**: 115 µg Pb/L **LOEC:** 128 µg Pb/L **MATC:** 121 µg Pb/L **EC_{10}:** 28 µg Pb/L **EC_{20}:** 55 µg Pb/L	Mebane et al. (2008)
Midge *(Chironomus riparius)*	Logarithmic range from 0 to 25,000 µg Pb/L (nominal)	24-hour acute toxicity tests with first-instar larvae exposed to Pb nitrate in synthetic soft water. *C. riparius* culture was from egg masses from Environment Canada.	Temperature: 20 °C Water hardness: 8 mg/L of $CaCO_3$,	**Survival:** Concentration-dependent decrease in survival with increasing Pb. Of the five metals tested in the study (Cd, Cu, Pb, Ni, Zn), Pb was most toxic to first instar *C. riparius*.	**24-hour LC_{50}:** 613 µg Pb/L	Béchard et al. (2008)

Table 6-5 (Continued): Recent evidence for Pb effects on freshwater plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Midge *(Chironomus javanus)*	430; 580,; 1,330; 2,460; 7,670 µg Pb/L (measured)	4-day exposure with fourth instar larvae to Pb nitrate in aerated, filtered, dechlorinated tap water with static aerated renewal at 2 days. Larvae were collected from a filter system of a fish pond in Bangi, Selangor, Malaysia.	Temperature: 28-30 °C pH: 6.51 ± 0.01 Conductivity: 244.3 ± 0.6 µS/cm DO: 6.25 ± 0.06 mg/L Total hardness (Mg^{2+} and Ca^{2+}): 15.63 ± 2.74 mg/L as $CaCO_3$	**Survival:** LC_{50} values reported for this species.	**24 hour LC_{50}:** 20,490 µg Pb/L **48 hour LC_{50}:** 6,530 µg Pb/L **72-hour LC_{50}:** 1,690 µg Pb/L **96 hour LC_{50}:** 790 µg Pb/L	Shuhaimi-Othman et al. (2011c)
Midge *(Culicoides furens)*	100; 290; 510; 800; 2,800 µg Pb/L (measured)	Series of 96-hour exposures to Pb chloride in dechlorinated water under several temperature ranges.	**1st experiment:** Temperature: 25-28 °C **2nd experiment:** Temperature: 20-26 °C **3rd experiment:** Temperature: 10, 15, 20, 23, 25, 28, 30, 35, 40 ± 0.5 °C	**Survival:** Higher and lower temperatures brought about increased toxicities. LC_{50} values generally increased in 10-25°C and decreased in 28-40°C. 40°C temperature produced 100% mortality.	**96 hour LC_{50} values:** **28-25 °C*:** 400 µg Pb/L **26-20 °C*:** 300 µg Pb/L **25 °C:** 400 µg Pb/L **35 °C-25 °C*:** 500 µg Pb/L **23 °C**: 700 µg Pb/L **20 °C**: 400 µg Pb/L **15 °C**: 400 µg Pb/L **10 °C:** 357 µg Pb/L *temperature decreased over the duration of the experiment	Vedamanikam and Shazilli (2008a)

Table 6-5 (Continued): Recent evidence for Pb effects on freshwater plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Midge *(Chironomus plumosus)*	3,000; 5,400; 8,200; 30,000; 54,000 µg Pb/L (measured)	Series of 96-hour exposures to Pb chloride in dechlorinated water under several temperature ranges.	**1st experiment:** Temperature: 25-28°C **2nd experiment:** 20-26°C **3rd experiment:** 10, 15, 20, 23, 25, 28, 30, 35, 40 ± 0.5 °C	**Survival:** Higher and lower temperatures brought about increased toxicities 40°C temperature produced 100% mortality LC_{50} values generally increased in 10-25°C and decreased in 28-40°C	**96 hour LC_{50} values:** **28-25°C***: 16,200 µg Pb/L **26-20°C*:** 8,300 µg Pb/L **25 °C:** 9,500 µg Pb /L **35 °C:** 700 µg Pb/L **30 °C:** 700 µg Pb/L **28 °C:** 900 µg Pb/L **25 °C:** 900 µg Pb/L **23 °C:** 700 µg Pb/L **20 °C:** 600 µg Pb/L **15 °C:** 600 µg Pb/L **10 °C:** 500 µg Pb/L *temperature decreased over the duration of the experiment	Vedamanikam and Shazilli (2008a)
Oligochaete worm *(Lumbriculus variegatus)*	1,300; 3,200; 8,000; 20,000; 50,000 µg Pb/L (nominal)	24 and 48-hour exposures to Pb nitrate spiked water from Lake Vesijärvi, Finland	Temperature: 20 °C	**Survival:** 48-hour LC_{50} reported	**48-hour LC_{50}:** 5,200 µg Pb/L	Penttinen et al. (2008)

Table 6-5 (Continued): Recent evidence for Pb effects on freshwater plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Mayfly *(Baetis tricaudatus)*	69; 103; 160; 222; 350; 546 µg Pb/L (measured)	96-hour static renewal test and 10 day chronic study with aerated Pb chloride spiked water, static renewal every 48 hours. Mayflies were collected from the South Fork Coeur d'Alene River, Idaho.	**Mean ± SD** Temperature: 9.3 ± 0.67 °C pH: 6.64 ± 0.18 Hardness: 20.7 ± 0.58 mg/L as CaCO3 Alkalinity: 19.8 ± 1.04 mg/L as CaCO3 Conductivity: 47.7 ± 1.72 µs DO: 10.1 ± 0.45 mg/L	**Growth:** Consistent dose-dependent reductions in mayfly growth (as number of molts); growth decreased with increased Pb exposure. **Survival:** 96-hour EC_{50} reported for this species. Reduced molting endpoint more sensitive than mortality endpoint.	**96-hour EC_{50}** 664 µg Pb/L **Survival:** **NOEC:** 222 µg Pb/L **LOEC:** 350 µg Pb/L **MATC:** 279 µg Pb/L **EC_{10}:** 169 µg Pb/L **EC_{20}:** 230 µg Pb/L **Molting :** **NOEC:** 103 µg Pb/L **LOEC:** 160 µg Pb/L **MATC:** 130 µg Pb/L **EC_{10}:** 37 µg Pb/L **EC_{20}**: 66 µg Pb/L	Mebane et al. (2008)
Mosquito *(Culex quinquefasciatus)*	**Acute test:** 100; 150; 200; 250 µg Pb/L (analytically verified) 50; 150; and 200 µg Pb/L for reproductive studies	24-hour acute toxicity test and several tests to assess reproductive endpoints. All tests were conducted with Pb nitrate in distilled water	Temperature: 25 ± 2 °C pH: 7	**Reproduction:** Hatching rate significantly decreased, lower emergence rates, larval development from L1 to adults took longer. **Survival:** 24 hour LC_{50} reported	**Survival:** **24 hour LC_{50}:** 180 µg Pb/L	Kitvatanachai et al. (2005)

Table 6-5 (Continued): Recent evidence for Pb effects on freshwater plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Neosho mucket *(Lampsilis rafinesqueana)*	Concentrations were measured and used to calculate EC_{50} values, reported in supplemental data.	24 and 48-hour exposure with 5 day old juveniles obtained from adults collected from Spring River, KS, U.S.	Temperature: 20 ± 1 °C pH: 7.2-7.6 DOC: >7.0 mg/L Hardness: 40-48 mg/L as $CaCO_3$ Alkalinity: 30-35 mg/L as $CaCO_3$	**Survival:** Neosho mucket is a candidate species for U.S. federal endangered and threatened status. Toxicity testing with newly transformed juveniles indicated that this species is sensitive to Pb exposure.	**24 hour EC_{50}:** 5 day old juveniles >507 µg Pb/L **48 hour EC_{50}:** 5 day old juveniles: 188 µg Pb/L	Wang et al. (2010f)

Table 6-5 (Continued): Recent evidence for Pb effects on freshwater plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Fatmucket mussel *(Lampsilis siliquoidea)*	For 28-day exposure: 0.04; 2.9; 6.1; 17; 36; 83 µg Pb/L (measured)	24 and 48-hour exposure with glochidia, 96-hour exposure with 5 day old, and 2 or 6 month old juveniles and 28-day exposure with 2 or 4 month old mussels in reconstituted soft water. Tests were conducted with glochidia and juveniles obtained from adults collected from the Silver Fork of Perche Creek, MO, U.S.	Temperature: 20 ± 1 °C pH: 7.2-7.6 DOC: >7.0 mg/L Hardness: 40-48 mg/L as $CaCO_3$ Alkalinity: 30-35 mg/L as $CaCO_3$	**Growth:** Growth of juvenile mussels in the 17 µg Pb/L concentration was statistically significantly reduced compared to growth in the controls at the end of 28 days. Growth was not assessed in the higher concentrations due to mortality. **Survival:** The 24-hour EC_{50} values for glochidia and 96-hour EC_{50} values for 2 and 6 month old juveniles were much higher than 96 hour LC_{50} value for 5 day old newly transformed juveniles. Genus mean chronic value was the lowest value ever reported for Pb. Survival was based on foot movement within a 5-minute observation period.	**24 and 48 hour EC_{50}**: glochidia >400 µg Pb/L (test 1) >299 µg Pb/L (test 2) **48 hour EC_{50}:** 5 day old juveniles 465 µg Pb/L (test 1) 392 µg Pb/L (test 2) **96 hour EC_{50}:** 5 day old juveniles 142 µg Pb/L (test 1) 298 µg Pb/L (test 2) **24 and 48 hour EC_{50}:** 2 month old juveniles >426 µg Pb/L **4 day EC_{50}:** >83 µg Pb/L **10 day EC_{50}:** >83 µg Pb/L **21 day EC_{50}:** 29 µg Pb/L **28 day EC_{50}:** 20 µg Pb/L **28-day NOEC** Juvenile fatmucket: 6.1 µg/L **28-day LOEC** Juvenile fatmucket: 17 µg/L **Genus mean chronic value** 10 µg Pb/L	Wang et al. (2010f)

Table 6-5 (Continued): Recent evidence for Pb effects on freshwater plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Snail *(Lymnaea stagnalis)*	4; 12; 16; 42; 113; and 245 µg Pb/L (measured)	30-day exposure with newly hatched snails (<24-hour old) in artificial fresh water with Pb nitrate.	Temperature: 23 ± 1 °C	**Growth:** Newly hatched snails exhibited greatly reduced growth in response to Pb exposure **Survival:** No Pb-induced mortality was observed.	**EC_{20}** <4 µg Pb/L **NOEC:** 12 µg Pb/L **LOEC:** 16 µg Pb/L	Grosell et al. (2006b)
Snail *(Lymnaea stagnalis)*	**1st Experiment:** <0.5 (control), 2.7 and 18.9 µg Pb/L (measured) **2nd Experiment:** 1.3 and 7.5 µg Pb/L (measured)	Pb exposures were performed with juvenile snails (~ 1 g) for 21 days and then 14 days in dechlorinated tap water under flow-through conditions.	Dechlorinated City of Miami tap water ($[Na^+]$ ~1.1 mmol/L $[Ca^{2+}]$ ~0.31 mmol/L $[Cl^-]$ ~1.03 mmol/L $[HCO^{3-}]$ ~0.68 mmol/L, [DOC]~200 µmol/L pH~7.7 at room temperature	**Growth:** In juveniles exposed to 18.9 µg/L Pb for 21 days, Ca^{2+} influx was significantly inhibited and model estimates indicated 83% reduction in growth of newly hatched snails after 30 days at this exposure concentration **Survival:** No Pb-induced mortality was observed		Grosell and Brix (2009)
Snail *(Marisa cornuarietis)*	5,000;10,000; and 15,000 µg Pb/L (nominal)	5-day, 6-day, and 10-day exposure to Pb chloride in deionized or double-distilled water. Snail strain used for egg production was from the Zoological Institute in Frankfurt, Germany.	Temperature: 24 ± 1 °C pH: ~7.5 Conductivity: ~800 µS/cm	**Reproduction:** Significant delay in hatching at 10,000 µg Pb/L **Growth:** Significantly delayed development (reduced visible tentacles, eye formation) at 15,000 µg Pb/L. No effect on fresh weight. **Survival:** Significantly increased mortality at 15,000 µg Pb/L	**LOEC:** 10,000 µg Pb/L	Sawasdee and Köhler (2010)

Table 6-5 (Continued): Recent evidence for Pb effects on freshwater plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Snail *(Biomphalaria glabrata)*	50; 100; and 500 µg Pb/L (nominal)	96-hour acute laboratory bioassays	Dechlorinated continuously aerated tap water: Temperature: 22 °C pH: 7.1 ± 0.2 Total hardness: 65 ± 3 mg $CaCO_3$/L Alkalinity: 29 ± 2 mg $CaCO_3$/L Conductivity" 230 ± 17 µS	**Reproduction:** Significant decrease in number of eggs laid at 500 µg Pb/L. **Survival:** Embryonic survival was 12% of the number of eggs laid by the control group at 100 µg Pb/L. Time to hatching increased 3 fold from the control. No embryos survived the highest concentration.		Ansaldo et al. (2009)
Prawn *(Macrobrachium lancesteri)*	22; 31; 48; 126; 170 µg Pb/L (measured)	4-day exposures in Pb chloride in aerated, filtered, dechlorinated tap water, static aerated, renewal at 2 days. Prawns were purchased from aquarium shops in Bani, Selangor, Malaysia.	Temperature: 28-30 °C pH: 6.51 ± 0.01 Conductivity: 244.3 ± 0.6 µS/cm DO: 6.25 ± 0.06 mg/L Total hardness (Mg^{2+} and Ca^{2+}): 15.63 ± 2.74 mg/L as $CaCO_3$	**Survival:** LC_{50} increased with decrease in mean exposure concentration	**24 hour LC_{50}** 85.9 µg Pb/L **48 hour LC_{50}:** 58.5 µg Pb/L **72 hour LC_{50}:** 45.5 µg Pb/L **96 hour LC_{50}** 35.0 µg Pb/L	Shuhaimi-Othman et al. (2011a)

Table 6-5 (Continued): Recent evidence for Pb effects on freshwater plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Crayfish (*Orconectes hylas)*	Reference sites: 0.03 µg Pb/L Mining sites: 0.12 to 1.59 µg Pb/L Downstream sites 0.03 to 0.04 µg Pb/L	in situ 28-day exposure with juvenile crayfish in streams impacted by Pb mining and reference sites in Missouri, USA.	Water quality parameters were measured at each site Temperature 23 to 26 °C pH: 7.9 to 8.1 Conductivity: 282 to 858 DO: 6.3 to 8.4 mg/L) Alkalinity 141 to 182 mg/L as $CaCO_3$ Turbidity: 0.4 to 0.6 NTU Sulfate: 0.3 to 304 mg/L Other metals were present downstream of mining sites	**Survival:** Crayfish survival and biomass were significantly lower in streams impacted by Pb mining Metal concentrations were negatively correlated with caged crayfish survival.		Allert et al. (2009a)

Table 6-5 (Continued): Recent evidence for Pb effects on freshwater plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Vertebrates						
Fathead minnow *(Pimephales promelas)*	**Measured: Mean ± SEM** Tap low Pb: 28 ± 1.1 µg Pb/L Tap high Pb: 105 ± 4.8 µg Pb/L HCO_3^- low Pb: 31 ± 1.2 µg Pb/L HCO_3^- high Pb: 113 ± 4.6 µg Pb/L Humic low Pb: 30 ± 1.4 µg Pb/L Humic high Pb: 112 ± 4.5 µg Pb/L	4-day, 10-day, 30-day, 150-day, and 300-day exposures in Pb nitrate spiked dechlorinated tap water with static renewal to study the effects of DOC and alkalinity on Pb toxicity. Breeding assays (21 days) were also performed.	Temperature: 22 ±1 °C **Tap H_2O:** Hardness: 91 mg/L pH: 8.1 **+500 µM $NaHCO_3$:** DOC: 257 µM Hardness: 93 mg/L pH: 8.3 **$NaHCO_3$:** Hardness: 93 mg/L pH: 8.3 **+4 mg/L HA:** Hardness: 93 mg/L pH: 8.0 **Humic:** Hardness: 93 mg/L pH: 8.0	**Growth:** No statistically significant growth differences observed at any age due to water chemistry alone; DOC addition strongly protected against Pb accumulation; increased alkalinity reduced whole body Pb burdens;. Growth inhibited at 4 days, but recovered by 30 days in high Pb concentration. **Reproduction:** HCO_3- reduced 21 day total reproductive output (reduced clutch size and number of clutches produced); addition of HCO_3^- alone actually increased reproductive output; significantly higher fecundity in HCO_3^- treatment; Egg attachment low in both tap water and HCO_3^- treatments; HA promoted attachment; No statistically significant differences in egg hatchability. HCO_3^- and humic acid treatments increased average egg mass; no effects on hatchability in the HCO_3^- and humic acid treatment;		Mager et al. (2010)

Table 6-5 (Continued): Recent evidence for Pb effects on freshwater plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Fathead minnow *(Pimephales promelas)*	<0.1 – 3,605 µg Pb/L (measured) Pb was quantified in fish tissues in a separate set of experiments.	30 day flow through exposure to Pb nitrate to determine to the effects of Ca^{2+}, humic acid and pH (6.3 and 8.3) on Pb accumulation and toxicity in juvenile fathead minnows.	Exposure media were made up from a base-water consisting of 2:1 deionized water: dechlorinated tap water. Temperature: 23 °C and had various levels of Ca^{2+}, humics, and pH values: 0.5; 1; 2 mM Ca^{2+} 2; 4; 8; 16 mg humic 6.3; 8.3 pH	**Growth:** No growth inhibition was observed in any treatment. An increase in growth was observed in groups exposed to higher Pb concentrations where there were high initial mortalities. **Survival:** For most treatments, mortalities occurred during the first 5 to 7 days of exposure. The lowest tolerance was observed at low pH (6.8). Addition of DOC or $CaSO_4$ decreased Pb toxicity.	**30 day LC_{50}, EC_{20}, and LOEC values*;**(low hardness basic test water [19 mg $CaCO_3$ /L], [Base water]) **LC_{50} in µg Pb/L:** Base water: 39 0.5 mM Ca^{2+}: 91 1.0 mM Ca^{2+}: 104 2 mg humic: 255 4 mg humic: 443 8 mg humic: 832 16 mg humic: 1903 pH 6.3: 4.5 pH 8.3: 13 **EC_{20} in µg Pb/L:** Base water: 22 0.5 mM Ca^{2+}: 47 1.0 mM Ca^{2+} 51 2 mg humic: 189 4 mg humic: 319 8 mg humic: 736 16 mg humic: 1729 pH 6.3: 2.1 pH 8.3: 8.7 **LOEC in µg Pb/L:** Base water: 62 0.5 mM Ca^{2+}: 40 1.0 mM Ca^{2+}: 107 2 mg humic: 199 4 mg humic: 475 8 mg humic: 919 16 mg humic: 1751 pH 6.3: 6.2 pH 8.3: 15 *4 day and 10 day LC_{50}, LC_{20}, and LOEC values also reported in the paper.	Grosell et al. (2006a)

Table 6-5 (Continued): Recent evidence for Pb effects on freshwater plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Fathead minnow *(Pimephales promelas)*	Measured but not reported. Figure 1 of Esbaugh et al. (2011) plots the relationship between dissolved and nominal Pb concentrations in three waters with low Pb solubility. Predicted concentration of major Pb chemical species in the natural water bioassays is provided in Table 4 of Esbaugh et al. (2011)	Acute toxicity of Pb to juvenile *P. promelas* (<24 hours old) was assessed in 96-hour static renewal exposures to two lab generated reference waters and seven natural waters from across North America selected to include a range of water quality parameters. Waters were spiked with varying concentrations of Pb as Pb nitrate.	Temperature: 26 °C Water chemistry of the field-collected waters are reported in Table 1 of Esbaugh et al. (2011) including pH (range 5.5 to 8.5), Ca^{2+} (range 24 to 1,934 µM), DOC (range 36 to 1,244 µM) and hardness (range 4 to 298 mg/L)	**Survival:** LC_{50} values ranged from 41 to 3,598 µg Pb/L. DOC had the strongest protective effect. The lowest LC_{50} occurred in the pH 5.5 water. No Pb toxicity was observed in three alkaline natural waters.	**96-hour LC_{50} range:** 41 to 3,598 µg Pb/L. **NOEC: range** 14 to 2,271 µg Pb/L. **LOEC: range** 42 to 5,477 µg Pb/L	Esbaugh et al. (2011)
Fathead minnow *(Pimephales promelas)*	0.2 ± 0.1 µg Pb/L (control) 33 ± 4 µg/L (chronic low) 143 ± 14 µg/L (chronic high) (measured)	33 to 57-day exposures in dechlorinated tap water and deionized water to Pb nitrate to assess swimming performance.	Temperature: 21 ± 1 °C pH: 7.50 ±0.03 Total CO_2: 543 ± 69 µmol/L DOC: 108 ± 4 µmol carbon/L Hardness: 26 ± 3 mg/L	**Growth:** Fish from the chronic low exposure were significantly larger than control fish (increased mass and body length).		Mager and Grosell (2011)

Table 6-5 (Continued): Recent evidence for Pb effects on freshwater plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Fathead minnow *(Pimephales promelas)*	Fish were feed *L. variegatus* exposed via water to 628 µg Pb/L.	Juvenile fish fed a live diet of the oligochaete *L. variegatus* for 30 days contaminated with Pb.	Water from Lake Superior that was subsequently filtered and UV-treated. Temperature: 25 °C pH: 7.5–8.0, Hardness: 45–50 mg $CaCO_3$, Alkalinity : 40-45 mg $CaCO_3$/L	**Growth**: Not significantly affected **Survival**: Not significantly affected		Erickson et al. (2010)
Zebrafish (*Danio rerio)*	Mean daily dietary dose of 0.417 (0.3-0.48) or 0.1 (0.07 – 0.14) mg Pb/kg per day (measured)	63 day dietary exposure with Pb-enriched polychaete, *Nereis diversicolor.* Adult zebrafish were fed a daily dose of 1% flake food (dry wet diet/wet weight fish), 1% brine shrimp, and 1% *N. diversicolor* collected from either Gannel Estuary, Cornwall, U.K., an estuary with legacy Pb contamination, or Blackwater Estuary, Essex, U.K., (reference site)	Temperature: 29 ± 1 °C	**Reproduction:** No impairment observed to incidence of spawning, numbers of eggs per breeding pair or hatch rate of embryos compared with pre-exposure levels. Metal analyses revealed significant increases in whole-body Pb burdens of male fish fed polychaetes from the contaminated estuary.		Boyle et al. (2010)

Table 6-5 (Continued): Recent evidence for Pb effects on freshwater plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Tilapia *(Oreochromis niloticus)*	Mean concentration of Pb in food pellets: 100; 400; and 800 mg Pb/kg dry weight (nominal)	Fish obtained from an unpolluted fish farm in Hangzhou, China were held in tanks with dechlorinated tap water and were fed diets with Pb nitrate twice daily for 60 days.	Temperature: 25 ± 1 °C pH: 7.1-7.5 DO: 7.5-7.8 mg/L Alkalinity: 109 mg $CaCO_3$/L Hardness: 118 mg $CaCO_3$ / L	**Growth:** No effects on growth were found. **Survival:** Exposure to Pb-contaminated diets did not result in mortality.		Dai et al. (2009b)
Channel catfish *(Ictalurus Punctatus)*	Fish were fed *L. variegatus* exposed via water to 576 µg Pb/L	Juvenile fish fed a live diet of the oligochaete *L. variegatus* for 30 days contaminated with Pb.	Water from Lake Superior that was subsequently filtered and UV-treated. Temperature: 25 °C pH: 7.5–8.0, Hardness: 45–50 mg $CaCO_3$/L Alkalinity: 40-45 mg $CaCO_3$/L	**Growth**: Not significantly affected **Survival**: Not significantly affected		Erickson et al. (2010)

Table 6-5 (Continued): Recent evidence for Pb effects on freshwater plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
African catfish (*Clarias gariepinus)*	100; 300; and 500 µg Pb/L (nominal) Pb was quantified in tissues following exposure.	24; 42; 90; 138; and 162 hour embryo exposure to Pb nitrate in dechlorinated tap water. These intervals corresponded to 30; 48,; 96; 144; and 168 hours post-fertilization.	Temperature: 24 °C pH: 8.0 Conductivity: 700 µs/cm Oxygen: 90-95% saturation	**Growth:** Malformations observed in exposed embryos (malformed embryos only survived shortly after hatching), delay in development. **Reproduction:** Concentration-dependent delay in hatching, reduced percentage of embryos completing egg stage period from 75% in control to 40% in 500 µg Pb/L.		Osman et al. (2007b)
Rainbow trout *(Oncorhynchus mykiss)*	**In diet:** 6.9 mg Pb per g dry mass) (*L. variegatus* exposed via sediments)	Juvenile fish fed a live diet of the oligochaete *L. variegatus* for 30 days contaminated with Pb of varying concentrations of Pb nitrate	Water from Lake Superior that was subsequently filtered and UV-treated. Temperature: 11 °C pH : 7.5–8.0 Hardness: 45–50 mg $CaCO_3$/L Alkalinity : 40-45 mg $CaCO_3$/L	**Growth**: Not significantly affected **Survival**: Not significantly affected		Erickson et al. (2010)

Table 6-5 (Continued): Recent evidence for Pb effects on freshwater plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Rainbow trout *(Oncorhynchus mykiss)*	Control Pb-free diet of 0.06 mg Pb/kg dry weight, and three different diets of 7; 77; and 520 mg Pb dry weight (0.02 (control), 3.7; 39.6; and 221.5 mg Pb/day dry weight calculated for food consumption) Pb also quantified in tissues	21-day exposure to juvenile rainbow trout via diet amended with Pb nitrate. Fish were held in aerated tanks with dechlorinated water	Temperature: 11-13 °C pH 7.5-8.0 Hardness: 140 ppm as $CaCO_3$	**Growth:** No effects on growth rates were observed in rainbow trout administered a diet containing three concentrations of Pb. Dietary Pb was poorly absorbed. Comparison of dietary and water-borne exposures suggest that toxicity does not correlate with dietary exposure, but does correlate with gill accumulation from waterborne exposure**.** **Survival:** Not significantly affected by dietary Pb		Alves et al. (2006)

Table 6-5 (Continued): Recent evidence for Pb effects on freshwater plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Rainbow trout *(Oncorhynchus mykiss)*	**ELS (early life stage) test 1:** 12; 24; 54; 143; and 384 µg Pb/L (measured) **ELS test 2:** 8; 18; 37; 87; and 124 µg Pb/L (measured)	96-hour static renewal acute toxicity test with swim-up stage fry (2 to 4 weeks post-hatch) reared from eggs used in the chronic studies. Two 60+ day ELS exposures were conducted in a flow-through system using temperature controlled water from Little North Fork of the South Fork Coeur d'Alene river in ELS 1 (69 days) and water from the South Fork in ELS 2 (62 days).	**ELS 1:** Temperature 9.8 ± 0.6 °C pH: 6.75 ± 0.4 Hardness: 19.7 ± 1.5 mg/L as $CaCO_3$ Conductivity: 45.8 ± 2.2 µs Alkalinity: 19.6 ± 2.2 mg/L as $CaCO_3$ DO: 10.2 ± 0.7 mg/L **ELS 2:** Temperature 12.5 ± 0.9 °C pH: 7.19 ± 0.3 Hardness: 29.4 ± 3.6 mg/L as $CaCO_3$ Conductivity: 69.1 ± 7.4 µs Alkalinity: 27 mg/L ± 2.1 as $CaCO_3$ DO: 9.2 ± 0.9 mg/L	**Growth:** In ELS 1, growth generally decreased as concentration increased, with fish in the highest surviving treatment (143 µg Pb/L) exhibiting severely stunted growth that was statistically different from the control. In ELS 2, growth increased in the highest treatment with a slight reduction in length and wet weight in intermediate exposures. **Survival:** In ELS 1 Survival decreased as concentration increased with complete mortality before the end of the test in the highest treatment (384 µg Pb/L). In ELS 2, survival decreased significantly in the highest treatment with high survival in intermediate exposure. In both tests, the greatest number of mortalities occurred around or shortly after the swim-up stage.	**96-hour LC_{50}:** 120 µg Pb/L **ELS 1: Survival** **NOEC:** 24 µg Pb/L **LOEC:** 54 µg Pb/L **MATC:** 36 µg Pb/L **EC_{10}:** 26 µg Pb/L **EC_{20}** : 34 µg Pb/L **ELS 1:Weight** **NOEC:** 24 µg Pb/L **LOEC:** 54 µg Pb/L **MATC:** 36 µg Pb/L **EC_{10}:** 39 µg Pb/L **EC_{20}:** 55 µg Pb/L **ELS 1: Length** **NOEC**: 54 µg Pb/L **LOEC:** 143 µg Pb/L **MATC:** 88 µg Pb/L **EC_{10}:** 64 µg Pb/L **EC_{20}:** 98 µg Pb/L **ELS 2: Survival** **NOEC:** 87 µg Pb/L **LOEC:** 125 µg Pb/L **MATC:** 104 µg Pb/L **EC_{10}:** 108 µg Pb/L **EC_{20}:** 113 µg Pb/L **ELS 2: Weight** **NOEC:** 37 µg Pb/L **LOEC**: 87 µg Pb/L **MATC:** 57 µg Pb/L **EC_{10}:** 7 µg Pb/L **EC_{20}:** >87 µg Pb/L **ELS 2: Length** **NOEC**: 8 µg Pb/L **LOEC:** 18 µg Pb/L **MATC:** 12 µg Pb/L **EC_{10}:** >87 µg Pb/L **EC_{20}:** >87 µg Pb/L	Mebane et al. (2008)

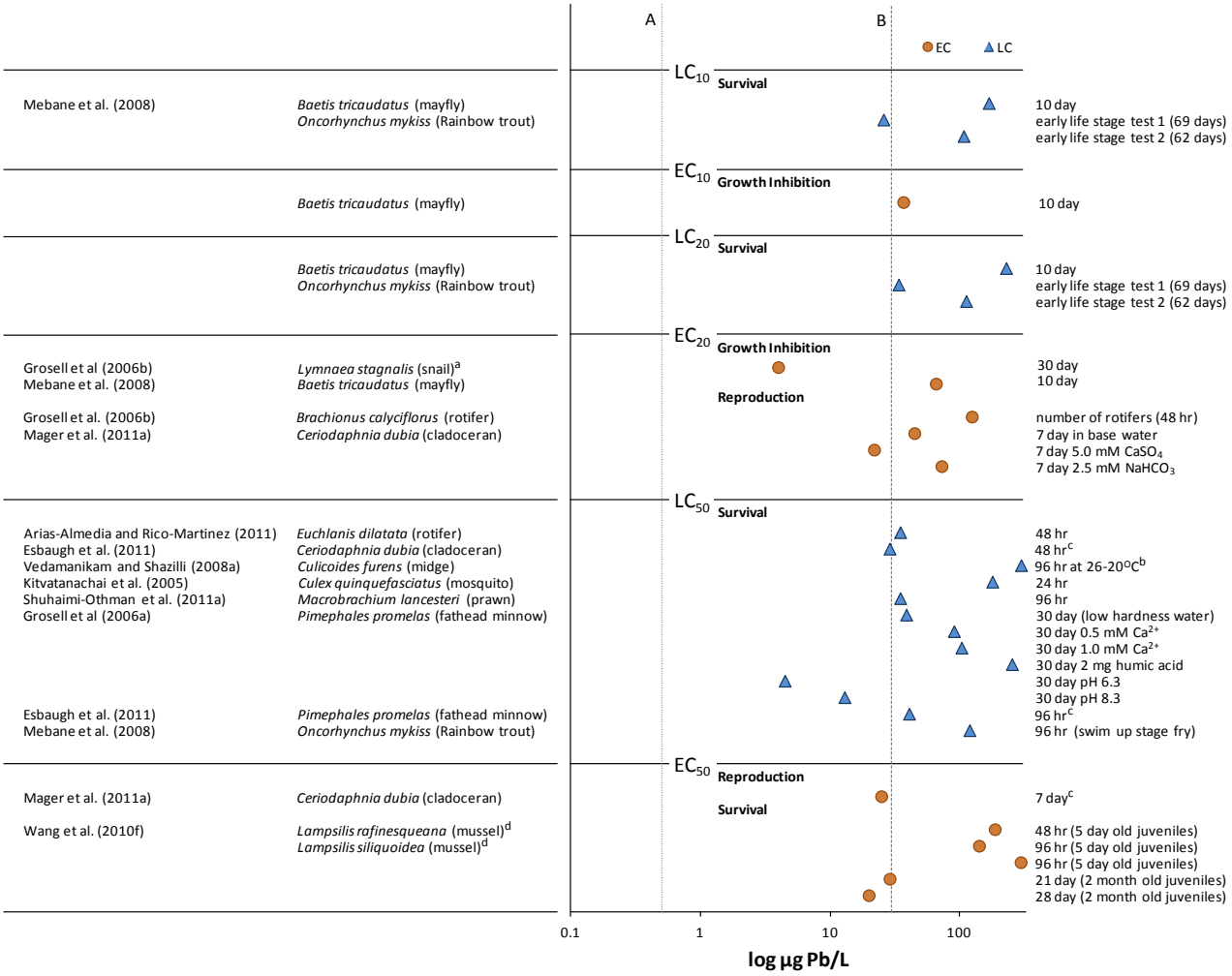
Table 6-5 (Continued): Recent evidence for Pb effects on freshwater plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Southern leopard frog (*Rana sphenocephala)*	Sediment: 45; 75; 180; 540; 2,360; 3,940; 5,520; 7,580 mg Pb/kg dry weight Corresponding sediment pore water: 123; 227; 589; 1,833; 8,121; 13,579; 19,038; 24,427 µg Pb/L (measured)	20; 40; 61; and 82-day exposures in Pb acetate spiked sediment collected from wetland, static renewal twice per week.	**Mean (SD)** Temperature: 21.6 °C pH: 6.92 DOC: 6.08 mg/L Conductivity: 168 µS/L Hardness: 7.30 mg Ca^{2+}/L Ammonia: 0.39 mg/L **Sediment:** Organic carbon: 8.25% Sand: 22.4% Silt: 38.4% Clay: 39.1%	**Growth:** Snout-vent length and body mass increased through time in all treatments; skeletal deformations (spinal deformations, digits truncated and twisted, long bones curved and truncated) increased with Pb content and length of exposure **Survival:** Exposure to ≥ 3,940 mg/kg sediment Pb (13,579 µg/L) pore water) killed all tadpoles within 5 days; tadpoles that reached climax stage (Gosner 42) had no difference in survival among treatments through the completion of metamorphosis.	**40 day EC_{50}** (for deformed spinal columns in sediment): 1,958 mg/kg Pb **Corresponding EC_{50}** (for deformed spinal columns in pore water): 6,734 µg/L **40 day EC_{50}** (for deformed spinal columns in sediment): 579 mg Pb/kg, and 1,968 µg Pb/L (in pore water). **Sediment: LC_{50}:** 3,728 mg Pb/kg (Corresponding to a **Pore water LC_{50} of:** 12,539 µg Pb/L).	Sparling et al. (2006)

Table 6-5 (Continued): Recent evidence for Pb effects on freshwater plants, invertebrates and vertebrates; growth, reproduction and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Northern leopard frog *(Rana pipiens)*	3; 10; and 100 µg Pb/L (nominal) (Pb was measured in tissues at the end of the study. Pb tissue concentrations ranged from 0.1 to 224.5 mg Pb/kg dry mass and fell within the range of tissue concentrations in wild-caught tadpoles).	Northern leopard frogs were exposed to Pb as Pb nitrate in dechlorinated water from the embryonic stage to metamorphosis (>66 days post-hatching)	Temperature: 21 to 22 °C pH 7.9 Hardness: 170 mg/L as $CaCO_3$	**Growth:** Tadpole growth was significantly slower in the early stages in 100 µg Pb/L treatment. More than 90% of tadpoles in the 100 µg Pb/L treatment developed lateral spinal curvature, whereas almost all the tadpoles in the other groups were morphologically normal. No significant effect of Pb exposure was found on percentage metamorphosis, snout–vent length, mortality, or sex ratio. Time to metamorphosis was delayed in 100 µg Pb/L treatment.		Chen et al. (2006b)

[a]References included are those which were published since the 2006 Pb AQCD.



[a]Reported as <4 µg Pb/L

[b]Temperature was decreased over the course of the exposure

[c]Reported as a range, lowest value shown

[d]Survival was assessed by foot movement.

Notes: Reference concentration "A" is the Median value of Pb reported from freshwater;
Reference concentration "B" is the Maximum value of Pb reported from freshwater.

Data in Figure 6-5 are limited to EC and LC values published since the 2006 Pb AQCD that are within one order of magnitude or less of the maximum value of Pb (30 µg Pb/L) reported for fresh surface water (minimum 0.04 µg Pb/L, median 0.5 µg Pb/L, 95th percentile 1.1 µg Pb/L) based on a synthesis of National Ambient Water Quality Assessment data collected from 1991-2003 [(U.S. EPA, 2006b); see Table 6-2].

Detailed summaries of the individual studies referenced above, including experimental conditions, form of Pb, modifying factors and responses at higher concentrations are summarized in Table 6-5.

Figure 6-5 Subset of concentration-response data reported in Table 6-5 for Pb effects on growth, reproduction, and survival in some freshwater invertebrates and fish.

Table 6-6 Recent evidence for Pb effects on saltwater plants, invertebrates, and vertebrates: growth, reproduction, and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Algae						
Microalgae (*Tetraselmis chuii, Rhodomonas salina, Chaetoceros* sp., *Isochrysis galbana, Nannochloropsis gaditana)*	50; 100; 250; 500; 800; 1,000; 1,600; 3,000; and 6,000 µg Pb/L, (nominal). Five nominal concentrations were analytically verified: 51; 225; 824; 1,704; 6,348 µg Pb/L (measured)	Populations of each microalgal species were exposed for 72 hours to ten progressively increasing nominal concentrations of Pb in filtered seawater	Temperature: 20 ± 1 °C pH 8.0	**Growth:** Growth inhibition (as measured by flow cytometry) was reported for each species. Species cellular size, sorption capacity, or taxonomy did not explain differences in sensitivity to Pb.	**EC_{50}:** *T. chuii:* 2,640 µg Pb/L *R. salina*: 900 µg Pb/L *Chaetoceros* sp.: 105 µg Pb/L *I. galbana*: 1,340 µg Pb/L *N. gaditana*: 740 µg Pb/L	Debelius et al. (2009)
Microalgae *(Tetraselmis suecica)*	20 µg Pb/L (nominal) *T. suecica* in this study was then fed to *Artemia franciscana* (mean Pb content 12 to 15 mg Pb/kg).	72-hour exposure to Pb nitrate in filtered natural seawater from Mazatlan Bay, Mexico. This was the first step in a four-level food chain.	Temperature: 28 ± 2 °C Salinity: 34.6 ± 1.2 ppt pH: 7.9-8.2 DO saturation: 90-95% (>7 mg/L)	**Growth:** Mean final cell concentrations, growth rate and total dry biomass were significantly reduced (40% lower than control cultures). Effects on primary, secondary and tertiary consumers were observed following Pb-exposure via *T. suecica* at the base of a simulated marine food chain.		Soto-Jiménez et al. (2011b)

Table 6-6 (Continued): Recent evidence for Pb effects on saltwater plants, invertebrates, and vertebrates: growth, reproduction, and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Invertebrates						
Polychaete *(Hydroides elegans)*	91; 245; 451; 4,443; 9,210; and 41,060 µg Pb/L (measured)	24-hour exposure of fertilized eggs to Pb chloride. Assay was stopped at 2 hours to assess effects on blastula.	Temperature: 27 ± 1 °C DO (86.5%) Salinity (34 ± 1 ppt) pH (8.1 ± 0.1) Carbonate 24.5 mg/L.	**Reproduction:** Exposure to Pb caused a significant decrease in the number of embryos developing normally to blastula after 2 to 3 hours of exposure to Pb.	**EC_{50}** Fertilization membrane stage : 30,370 µg Pb/L Blastula 1,429 µg Pb/L 24 hour trochophore larva: 231 µg Pb/L.	Gopalakrishnan et al. (2007)
Polychaete *(Hydroides elegans)*	48; 97; 201; 407; 803; 1,621 µg Pb/L (measured)	A series of experiments were performed from 20 minutes to 4 days in Pb chloride using polychaetes collected from seawater in Chennai, India.	Temperature: 27 ± 1 °C DO: 7–9 mg/L Salinity 34 ± 1ppt pH: 8.1 ± 0.1 Carbonate: 22.5 mg/L	**Reproduction:** Fertilization rate decreased by 70% in sperm pretreated with 97 µg Pb/L for 20 minutes. Fertilization rate of eggs pretreated in 48 µg Pb/L decreased to 20% of control. Life stages of *H. elegans* varied in their sensitivity to Pb. Gametes, embryo and larvae were more sensitive than adults with the larval settlement period being most sensitive to Pb exposure.	**EC_{50}** Sperm toxicity: 380 µg Pb/L Egg toxicity: 692 µg Pb/L Embryo toxicity: 1,130 µg Pb/L Blastula to trochophore: 261 µg Pb/L Larval settlement: 100 µg Pb/L	Gopalakrishnan et al. (2008)
				Survival: LC_{50} reported for adults	**Adult 96-hour LC_{50}** : 946 µg/L	

Table 6-6 (Continued): Recent evidence for Pb effects on saltwater plants, invertebrates, and vertebrates: growth, reproduction, and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Polychaete (*Capitella* sp.)	85; 137; 251; 392; 487; 738; 871 mg Pb/kg (measured)	3 and 6-day exposure for growth experiments, 96-hour exposure to Pb chloride spiked sediment from Chi-kou Estuary, Taiwan	Aerating circulating seawater Temperature: 20 ± 2 °C Salinity: 30%	**Growth:** Significant differences among growth rates of *Capitella* sp. in different levels of Pb-contaminated sediments, with the exception of 251 mg/kg treatment in the 6-day experiment. Growth rates deceased significantly from the control in the 3-day experiment but changes were inconsistent with increasing Pb concentration. **Survival**: No effect	**Growth LOAEL**: 85 mg Pb/kg	Horng et al., (2009)
Amphipod (*Elasmopus laevis*)	30-mg Pb/kg (control whole-sediment), 58 mg Pb/kg; 118 mg Pb/kg; 234 mg Pb/kg; 424 mg Pb/kg (measured)	Multi-generational bioassay with amphipods collected in Jamaica Bay, New York exposed 60+ days to sediment spiked with Pb acetate in filtered seawater. 10-day and 28-day bioassays were also conducted.	Temperature: 19-24 °C Salinity: 27-29 g/L DO: >6.57 mg/L	**Reproduction:** Fecundity was reduced as sediment Pb concentration increased. Onset of reproduction and reproduction were delayed as Pb concentration increased. **Survival:** No differences in adult survival among the Pb concentrations tested in 28-day and 60-day exposures.	Fecundity and time of first offspring was significantly reduced with increasing sediment concentration above 118 mg Pb/kg. Onset to reproduction significantly delayed at 118 mg Pb/kg and delayed further at higher tested concentrations.	Ringenary et al. (2007)

Table 6-6 (Continued): Recent evidence for Pb effects on saltwater plants, invertebrates, and vertebrates: growth, reproduction, and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Amphipod *(Melita plumulosa)*	Water-only tests ranged from 0 to 4,000 µg Pb/L (analytically verified) **Adult Sediment Test:** 500; 1,000; 2,000; 4,000 mg Pb/kg dry weight (analytically verified) **Juvenile Sediment Test:** 500; 1,000; 2,000 mg Pb/kg dry weight (analytically verified)	Juveniles and adults were tested in 96-hour seawater only or 10 day static-non-renewal exposure spiked sediment collected from intertidal mud flats, Woronora River, New South Wales, Australia. Adults were also tested in 10-day seawater only exposures.	Temperature 21 ± 1 °C, Salinity 30 ± 1%, pH 7.2–8.2, Ammonia (total) <3 mg N/L	**Survival:** Juvenile amphipods were more sensitive to Pb than adults in seawater and sediment exposures.	**96-hour seawater-only** **Adults:** LC_{50} 3,000 µg Pb/L NOEC 850 µg Pb/L LOEC 1,680 µg Pb/L **Juveniles:** LC_{50} 1,530 µg Pb/L NOEC 400 µg Pb/L LOEC 600 µg Pb/L **Seawater-only 10 days:** **Adults:** LC_{50} 1,270 µg Pb/L NOEC 190 µg Pb/L LOEC 390 µg Pb/L **10 days Sediment-only** **Adults:** LC_{50} NOEC, LOEC >3,560, mg Pb/kg **Juveniles:** LC_{50} 1,980 mg Pb/kg NOEC 580 mg Pb/kg LOEC 1,020 mg Pb/kg	King et al. (2006)
Brine shrimp *(Artemia franciscana)*	Mean Pb content 12 to 15 mg Pb/kg from dietary exposure to Pb.	This was the second step in a four-level food chain. *A. franciscana* feeding on *Tetraselmis suecica* cultured in 20 µg/L Pb, as Pb nitrate.	Temperature: 28 ± 2°C Salinity: 34.6 ± 1.2 ppt pH: 7.9-8.2 DO saturation: 90-95% (>7 mg/L)	**Growth:** A tendency toward lower biomass yields was reported (significant only on day of final harvest). **Survival:** A tendency toward lower survival was reported (significant only on day of final harvest).	Dry biomass was 195 mg/L in control cultures and 153 mg/L in cultures fed Pb exposed *T. suecica*. Mean cell count (individuals/L) on days 19-23 (harvest) was 320 in control and 255 in *A. franciscana* cultures fed Pb-exposed *T. suecica*.	Soto-Jiménez et al. (2011b)

Table 6-6 (Continued): Recent evidence for Pb effects on saltwater plants, invertebrates, and vertebrates: growth, reproduction, and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Shrimp *(Litopenaeus vannamei)*	Pb in exoskeleton, hepatopancreas, muscle, and remaining tissues was quantified on days 0; 15; 28; and 42 of the dietary study	This was the third step in a four-level food chain. *L. vannamei*, fed *A. franciscana* (mean Pb content 12 to 15 mg Pb/kg) feeding on *T. suecica* cultured in 20 µg/L Pb as Pb nitrate.	Temperature: 28 ± 2°C Salinity: 34.6 ± 1.2 ppt pH: 7.9-8.2 DO saturation: 90-95% (>7 mg/L)	**Growth:** Tendency toward decreased total length and weight was reported (significant only on day of final harvest)	Total mean length in the shrimp fed the experimental diet was 13 mm and wet weight was 7.1 g compared to mean total length (14.8 mm) and wet weight (8.5 g) at day 42.	Soto-Jiménez et al. (2011b)
				Survival: Tendency toward lower survival was reported (significant only on day of final harvest)	Mean survival in the shrimp fed the experimental diet ranged from 67 to 78% compared to control survival (84 to 90%) at day 42.	
Sea urchin *(Paracentrotus lividus)*	50 to 5,000 µg Pb/L (nominal)	Gametes and embryos exposed 48 to 50 hours in filtered seawater to Pb nitrate from adults collected from the Bay of Naples, Italy.	Filtered sea water Temperature: 18 ± 1 °C Salinity: 38% pH: 8 ± 0.2	**Growth:** Up to LOEC concentration, defects observed in plutei were mainly reduction in size (20%); above LOEC concentration, developmental defects were mainly larvae affected in skeletal or gut differentiation up to 2,000 µg Pb/L, where arrest of development started to increase.	**EC_{50}** 1,250 µg Pb/L **NOEL** 250 µg Pb/L **LOEC** 500 µg Pb/L	Manzo et al. (2010)
Mussel *(Mytilus galloprovincialis)*	3,500; 4,500; 5,500; 6,000 µg Pb/L (nominal)	24-hour static aerated exposure in seawater with Pb acetate with mussels collected from a mussel farm in Greece.	Temperature: 25 ± 2°C Salinity: 35% DO: 7-8 mg/L	**Survival:** Mortality at high Pb concentrations	**24-hour LC_{50}** 4,500 µg Pb/L	Vlahogianni and Valavanidis (2007)
Clam *(Meretrix meretrix)*	2; 20; 197; 1,016; 7,158 µg Pb/L	24-hour and 96-hour toxicity test with Pb nitrate in	Temperature: 28 ± 1°C Salinity:	**Reproduction:** Embryo development inhibited by increasing Pb concentrations	**Embryogenesis EC_{50}**: 297 µg Pb/L	Wang et al. (2009d)

Table 6-6 (Continued): Recent evidence for Pb effects on saltwater plants, invertebrates, and vertebrates: growth, reproduction, and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
	(measured)	filtered seawater using gametes from adults collected from Wenzhou, China and held under laboratory conditions.	20% pH: 7.8	**Growth:** Significant concentration-dependent growth inhibition in larvae. Larval metamorphosis rate decreased, no adverse effect on larval settlement at 20.4 µg Pb/L	**Growth:** **EC_{50}:** 197 µg Pb/L **Metamorphosis**: >7,160 µg Pb/L	
				Survival: Significant concentration-dependent survival inhibition of embryos over time	**48-hour LC_{50}:** >7,160 µg Pb/L **96-hour LC_{50}**: 353 µg Pb/L	
Scallop *(Argopecten ventricosus)*	For growth: 10; 100; 1,000; and 10,000 µg/L (analytically verified)	144 hour (survival) or 30 day (growth) exposure to Pb nitrate (static renewal every 48 hours) with juvenile *A. ventricosus* hatched from laboratory cultures held at Universidad Autonoma de Baja California Sur, Mexico.	Temperature: 20°C Salinity: 36 ± 1% DO: >4 mg/L	**Growth:** Juvenile growth rates and weight were significantly reduced at high concentrations of Pb	**EC_{50} for growth**: 4,210 µg Pb/L	Sobrino-Figueroa et al. (2007)
	For survival: 280; 560; 1120; 2,250; and 5,000 µg/L (analytically verified)			**Survival:** Juvenile mortality was significantly different than control at 96 hour LC_{50}	**72-hour LC_{50}:** 4,690 µg Pb/L **96-hour LC_{50}**: 830 µg Pb/L **120-hour LC_{50}**: 680 µg Pb/L **144-hour LC_{50}**: 680 µg Pb/L	

Table 6-6 (Continued): Recent evidence for Pb effects on saltwater plants, invertebrates, and vertebrates: growth, reproduction, and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Bivalve *(Tellina deltoidalis)*	1,000 mg Pb/kg (analytically verified)	10 day direct exposure to Pb nitrate spiked sediment collected from Woronora River, New South Wales, Australia. Adults used in the test were collected from Lane Cove River, Sydney, Australia and held in filtered seawater.	Temperature: 21 ± 1 °C Salinity 30 ± 1%, pH 7.2–8.2 Ammonia (total) <3 mg N/L	**Survival:** All individuals survived.	**10 Day NOEC** Porewater: 15 µg Pb/L dissolved Pb Sediment: 1,000 mg Pb/kg	King et al. (2010)
Vertebrates						
Toadfish *(Tetractenos glaber)*	Measured but not reported	Field-collected fish-Pb sampled from sediments collected in two reference and two metal contaminated estuaries near Sydney, Australia	Temperature : 15 to 16 °C Salinity: 29 to 31%, pH: 8.4 to 8.6, amongst estuaries. Sediment pH: 7.0-7.5 Organic matter: 1.5-2.1%	**Reproduction:** Decreased oocyte diameter and density in the toadfish were associated with elevated levels of Pb in the gonads of field collected fish; authors state that this is suggestive of a reduction in egg size.	N/A	Alquezar et al. (2006)

Table 6-6 (Continued): Recent evidence for Pb effects on saltwater plants, invertebrates, and vertebrates: growth, reproduction, and survival.

Species	Concentration	Exposure Method	Modifying factors	Effects on Endpoint	Effect Concentration	Reference[a] (Published since the 2006 Pb AQCD)
Grunt fish *(Haemulon scudderi)*	Mean total Pb body burden increased from 0.55 to 3.32 mg Pb/kg during the feeding experiment.	42-day dietary exposure from simulated marine food chain-shrimp, *L. vannamei*, fed *A franciscana* (mean Pb content 12-15 mg Pb/kg) feeding on *T. suecica* cultured in 20 µg/L Pb as Pb nitrate.	Temperature: 28 ± 2 °C Salinity: 34.6 ± 1.2 ppt pH: 7.9-8.2 DO saturation: 90-95% (>7 mg/L)	**Survival:** No significant differences observed in intermediate and final length, mean wet weight, or Fulton's condition factor: Final survival significantly lower; mean total Pb body burden increased.	Mean survival in the fish fed the experimental diet ranged from 65 to 75% compared to control diet survival (88 to 91%).	Soto-Jiménez et al. (2011b)

[a]References included are those which were published since the 2006 Pb AQCD.

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